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Microcalorimetric Study of the Temperature-Induced Phase Separation in Aqueous Solutions of Thermosensitive Polymers

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Microcalorimetric Study of the Temperature-Induced Phase Separation in Aqueous Solutions of Thermosensitive Polymers

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Mémoire accepté le : .....

Résumé :

Certains polymères, tels que le poly(vinylméthylether), l'hydroxypropylcellulose, le poly(N-isopropylacrylamide) (PNIPAM) et bien d'autres sont solubles dans l'eau froide mais précipitent lorsque leurs solutions sont chauffées. Cette propriété est utilisée dans des applications industrielles et présente également un très grand intérêt scientifique. Dans ce projet, les solutions aqueuses de deux polymères thermosensibles, la poly(2-isopropyl-2-oxazoline) et le poly(N-vinylisobutyramide), ont été étudiées par microcalorimétrie et turbidimétrie dans le but de connaître les paramètres thermodynamiques contrôlant leur transition de phase. L'influence de la masse molaire des polymères sur leur solubilité dans l'eau a été examinée en fonction de la temperature. En plus, les propriétés de ces polymères ont été comparées à celles de PNIPAM dont la structure est très proche de celles de poly(2-isopropyl-2-oxazoline) et de poly(N-vinylisobutyramide).

Dans ce but, trois techniques ont été utilisées :

1- La calorimétrie thermique différentielle (DSC).

2- La calorimétrie par perturbation de pression (PPC).

3- La turbidimétrie (UV-visible).

La DSC nous a permis de déterminer la température de séparation de phase, l'enthalpie de la transition de phase ainsi que d'autres paramètres thermodynamiques; d'autres part la PPC a été utilisée pour mesurer le coefficient d'expansion thermique du polymère et le changement du volume d'hydratation du polymère durant la transition de phase. En outre, la turbidimétrie a servi pour la détermination du point de trouble des solutions de polymères. Cette étude révèle que ces polymères possèdent différentes

propriétés physiques malgré qu'ils ont des structures similaires, ceci est du au fait que leur interaction moléculaire avec l'eau diffère.

Mots- clés : Solubilité, interactions moléculaires, point de trouble, température de transition, volume d'hydratation, calorimétrie, poly(N-vinylisobutyramide), poly(2-isopropyl-2-oxazolines).

Abstract

Polymers, such as poly(vinylmethylether), hydroxypropylcellulose, poly(N-isopropylacrylamide) (PNIPAM) and others are soluble in cold water, but they precipitate when the solution is heated. This property is used in industrial applications and it becomes a topic of major scientific interest. In this project, two thermosensitive polymers, the poly(2-isopropyl-2-oxazolines) and the poly(N-vinylisobutyramide) have been studied by microcalorimetry in order to determine the thermodynamic parameters that control the solubility of these polymers in water. The effect of the molecular weight of the polymer on their solubility was also examined. The properties of polymers were compared with those of PNIPAM since their chemical structures are closely related.

To do so, three techniques were used:

1- Differential scanning calorimetry (DSC).

2- Pressure perturbation calorimetry (PPC).

3- Turbidity measurment (UV-Visible).

DSC allowed us to determine the phase separation temperature, the enthalpy of the phase transition as well as other thermodynamic parameters. PPC was used to measure the thermal expansion coefficient of polymers and the changes in the volume of the hydration layer of the polymers. Moreover, the UV-visible was utilised for the determination of the turbidity points of the polymer solutions. Our studies reveal that these p olymers h ave different physical properties whereas they have similar structures, this could be due to their different molecular interactions with water.

Keywords: Solubility, molecular interactions, cloud point, transition temperature, hydration volume, calorimetry, poly(N-vinylisobutyramide), poly(2-isopropyl-2-oxazolines).

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Abbreviations list

DSC	Differential scanning calorimetry
PPC	Pressure perturbation calorimetry
HS DSC	High-sensitivity differential scanning microcalorimetry
PIPOZ	Poly(2-isopropyl-2-oxazolines)
NaCl	Sodium chloride
D_2O	Deuterium oxide
H_2O	Water
PNIPAM	Poly(N-isopropylacrylamide)
PNVIBA	Poly(N-vinylisobutyramide)
PEG	Poly(ethylene glycol)
PEOZ	Poly(2-ethyl-2-oxazolines)
PMOZ	Poly(2-methyl-2-oxazolines)
M_n	Number average molecular weight
M_w	Average molecular weight
PDI	Polydispersity indice
NMR	Nuclear magnetic resonance
MALDI-TOF	Matrix assisted laser desorption ionization time of flight
GPC	Gel permeation chromatography
RAFT	Radical addition fragmentation transfer
UV-Vis	Ultraviolet-visible
DMF	Dimethyl formamide

LiCl	Lithium chloride
THF	Tetrahydrofuran
$CDCl_3$	Chloroform-d-
t	Triplet
m	Multiplet
d	Doublet
ppm	Part per million
h	Hour
δ	Chemical shift in NMR
λ	Wavelength
α	Thermal expansion coefficient
n	Number of repeat unit
m/z	Mass over charge in MALDI-TOF
C_p	Heat capacity
T_{onset}	Temperature of the onset of the transition
T_{max}	Maximum temperature of transitions
ΔH	The enthalpy of the transition
ΔV	The change in the solvation layer of the polymer
$\Delta V/V$	The volume change in percent of the total polymer volume

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Acknowledgments

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Last, but certainly not least, I am grateful to my parents for their permanent support, prayers and encouragement. Without you, this would not have been possible.

CHAPTER 1

Introduction

1. The interactions properties of aqueous polymer solutions:

1.1. Introduction

The dissolution of polymers in water is a complex phenomenon that involves a number of weak interactions, such as the hydrogen bond formation between water and functional groups linked to the polymer, van der Waals interactions, and in the case of polyelectrolytes, ionic interaction between the charged units of the polymer, their counter ions, and additional salts under some situations. These interactions act together to facilitate, or prevent, the dissolution of a polymer. The physicochemical characteristics of these interactions are reviewed in the next section.

1.2 Hydrogen bonding

Hydrogen bond occurs when two electronegative atoms, such as nitrogen and oxygen, interact with the same hydrogen atom as shown in this figure below.

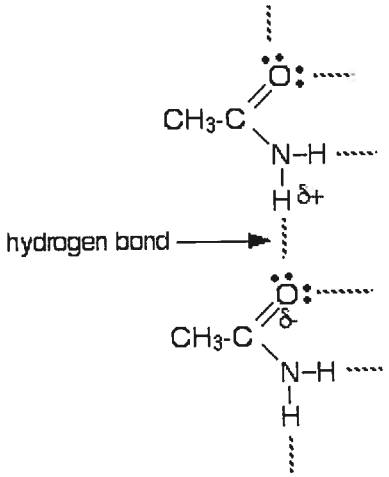

Figure 1 : Example of hydrogen bond between a hydrogen atom and nitrogen or oxygen atoms.

The hydrogen atom is normally covalently attached to one atom, the *donor*, but interacts electrostatically with the other, the *acceptor*. This interaction takes place between the dipoles of the electronegative atoms and the proton. One useful picture of a hydrogen bond is electrostatic, an attraction between the positive end of a dipole on one molecule and the negative end of a dipole on another. The more polar the molecule involved, the greater the degree of positive and negative charge associated with the dipoles, and the stronger the hydrogen bond, as depicted in figure 2 in the case of water and ammonia. Hydrogen bond (~20 $kJmol^{-1}$) is a weak [compared to covalent bonds (~465 $kJmol^{-1}$)] attraction between the unshared electron pairs on nitrogen or oxygen in one molecule and on hydrogen (covalently bonded to nitrogen or oxygen) on another.

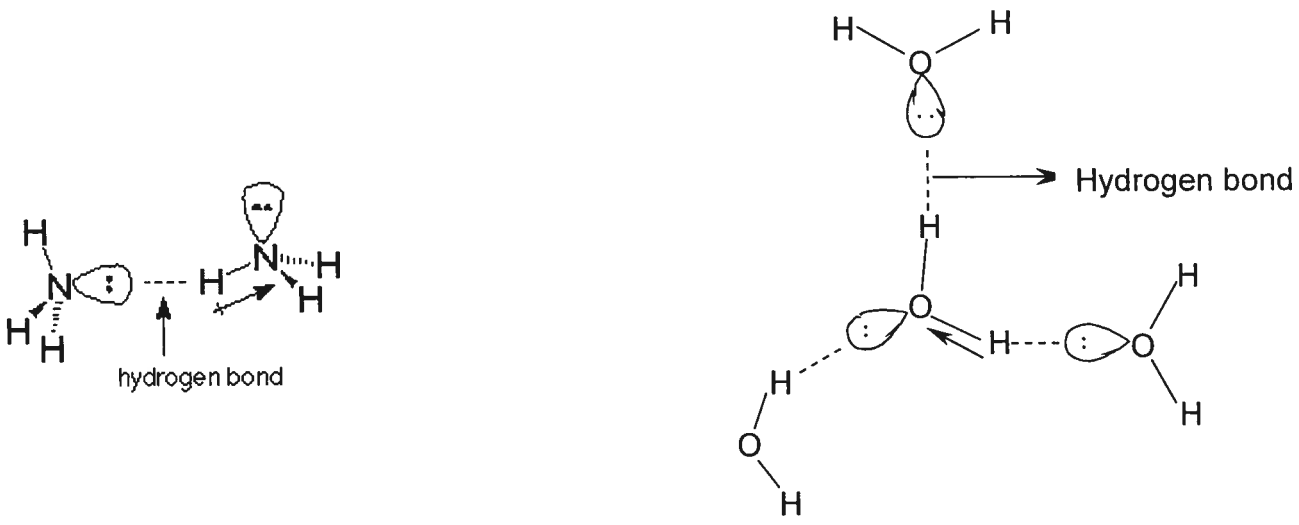

Figure 2 : Hydrogen bonds formed in ammonia (left) and water (right).

1.3 Hydrophobic interactions

Another very important interaction, which takes place when amphiphilic polymers are placed in contact with water, is the interaction between nonpolar substituents of the amphiphile that cannot form H-bonds with H_2O. These groups are hydrophobic (water hating), as opposed to "water loving", hydrophilic. Hydrophobic molecules or groups tend to aggregate to avoid H_2O molecules. Hydrophobic interactions are clearly demonstrated when one puts an oil drop in water. In fact, when a nonpolar molecule is placed in water the hydrogen bonding network of water is disrupted, the water molecules therefore reorganize around the solute in order to gain back free energy loss due to the loss of hydrogen bonds. This reorganization results in a considerable loss in the entropy of water.

1.4 Cloud point of aqueous polymer solutions

A large number of aqueous polymer solutions exhibit a heat-induced phase separation in water. This can be visualized readily in most cases: a clear polymer solution suddenly becomes milky when it is heated to a specific temperature. This temperature is known as the lower critical solution temperature (LCST) or sometimes clouds point (CP). This property has been investigated by several research groups with various polymers [1,2]. They reported that aqueous solutions of polymers exhibit a cloud point temperature that depends on the structure of the polymer, its molecular weight [3] and the concentration of the solution [4].

A homogeneous polymer solution is obtained when the free energy of mixing $\Delta G \leq 0$, so that the free energy of the solution is lower than the Gibbs energy of the mixture of water and the polymer. The relationship between the free energy, the enthalpy and the entropy is given by the thermodynamic equation below:

$\boldsymbol{\Delta G = \Delta H - T\Delta S}$ where *G is the Gibbs free energy, H the enthalpy, T the temperature, and S the entropy.*

Below the LCST, the dissolution of the polymer in water is favoured as a result of hydrogen bonding between water molecules and the polymer chains. The formation of hydrogen bonds lowers the free energy ΔG of the solution; however, the specific molecular orientations required by these bonds lead to negative entropy changes ΔS and consequently positive contributions to the free energy leading to phase separation above the LCST when the enthalpic contribution ΔH to the free energy is dominated by the growing entropic component [5].

1.5 Factors affecting the cloud point

1.5.1 Polymer properties

1.5.1.1 Polymer structure

The LCST of a polymer in aqueous solution can be controlled by varying its chemical composition, namely changing the fraction of the hydrophilic groups and hydrophobic groups, by changing the polymer concentrations or molecular weight. For example, linear polymers such as linear polyesters were synthesized from monomers,

which include hydrophilic ethylene oxide units and hydrophobic ethylene units. Controlling the fractions of the hydrophilic monomer and hydrophobic monomer in the polymer, the LCST of the polymer in solution may be controlled over a wide temperature range[6]. The phase separation temperature of the aqueous solution can be controlled, also, by varying not only the ratio of hydrophilic- hydrophobic components, but also their distribution along the chain.

1.5.1.2 Polymer molecular weight

Many researchers have reported the molecular weight influence; depending on the polymer, there exists an inverse dependence of the LCST on the molar masses [3, 7], but the opposite may take place as well [8]. Moreover, studies reported that the molecular weight had no influence on the LCST [9, 10]. In the study presented in this thesis, it will be demonstrated that the molecular weight affects enormously the LCST of the polymers studied.

1.5.2 Solvent properties

A number of parameters have been reported to influence the hydrogen bonding and the hydrophobic interactions in aqueous solution of polymers, thereby influencing the LCST of aqueous polymer solutions. Presented in this section, are the effect of solvent and of added salt.

1.5.2.1 Effect of the solvent

The LCST of aqueous polymer solutions changes when one replaces H_2O by D_2O [7]. Heavy water is known to have similar properties as light water, but there are some subtle differences between the two liquids making them an ideal pair of liquids for testing

various theories. Some of the physical properties of D_2O and H_2O are listed in Table 1. Briefly, D_2O is denser and more viscous than H_2O and has higher melting and boiling points. Differences in other physical properties are less marked. Deuterium bonds in D_2O are stronger than the analogous hydrogen bonds in H_2O.

Property	**D_2O**	**H_2O**
Melting point (°C)	3.82	0
Boiling point (°C)	101.72	100.0
Density (20°C, g/mL)	1.1056	0.9982
Temp. of maximum density (°C)	11.6	4.0
Viscosity (20°C, centipoise)	1.25	1.005
Surface tension (25°C, dyn·cm)	71.93	71.97
Heat of fusion (cal/mol)	1,515	1,436
Heat of vaporization (cal/mol)	10,864	10,515

Table 1: Physical properties of D_2O and H_2O.

Note: Surface tensions and dielectric constants of H_2O and D_2O are essentially identical.

1 poise = 0.1 Pa·s; 1 dyn = 10 mN; 1 cal = 4.1858 J.

In general, D_2O has two effects on polymer solutions. One is a "solvent effect," because of the properties of D_2O itself, and especially its effects on the structure of water and macromolecules. The second results from the ability of D_2O to replace H with D in

molecules. The first effect, which is mostly the effect of hydrogen bonding, was studied for the poly(N-isopropylacrylamide) (PNIPAM) in D_2O and H_2O [2]. The LCST of PNIPAM was found to be higher in D_2O compared to H_2O. The underlying reasons for this increase are not precisely known but it has been observed that polymer chain in D_2O solutions take on more extended conformations than with H_2O[11]. Furthermore, the amide bond of the PNIPAM is more exposed to water below the LCST. Moreover, the hydrogen bond is 5% stronger in D_2O than H_2O [12] . so that breaking the hydrogen bonds between water molecules and amide moieties will be more costly enthalpically in D_2O, thus the LCST will occur at higher temperature.

1.5.2.2 Effect of added salt

The presence of salts in the solutions may decrease or increase the LCST [4, 7, 13, 14, 15]. The salt effect on the LCST of thermosensitive polymers is due to salt induced changes of the water structure. Some salts increase the LCST of the polymers, a phenomenon called "salting-in" effect, while other salts decrease it, the "salting-out effect". Most of the inorganic salts have shown to trigger a salting out (water structure maker) effect on the solution of the polymers [10]. Salting-out is believed to be due to the removal of water associated with the polymer by the salt. Salting-out has been explained also by the fact that work is required for an organic molecule of low dielectric constant to come between such ions as Na^+ and Cl^-, which have no specific interaction with the molecule [16]. This effect was dependent on both cations and anions. von Hippel and coworkers [17] have reported that cations and anions are effective in altering the cloud point of a solution of poly(ethylene oxide) substituted

with methoxypoly(ethylene glycol) and glycine ethyl ester in the order of salting-in to salting-out effect:

$$\mathbf{Li^+ > NH_4^+ > K^+,\ Na^+ \text{ and } ClO_4^- > Br^- > Cl^- > F^- > CO_3^{2-} > SO_4^{2-}}$$

Similarly, Horne and coworkers [18] have investigated the salt effect on the cloud point of the PEO system, and they found that the order of the salting-in and salting-out effect of the cations and anions was almost the same as the above results. In fact, an increase in the concentration of a water structure breaker salt, such as NaI, induces an increase in hydrogen bonding between molecules of water and the polymer, resulting in an increase of the LCST. On the contrary, water structure makers such as NaCl, decrease the interaction of water with polymers, resulting in a decrease of the LCST.

The effect of organic salts on the LCST depends on both cations and anions. The larger cations have a more pronounced salting-in effect; they enhance the hydrogen-bonded structure because of their very hydrophobic nature. Such cations can bind to the hydrophobic part of the polymer, resulting in an increase in the hydrophilicity of the polymer molecules [19]. For example, in the case of tetraalkylammonium halides, the chain length of their alkyl groups plays an important role: an increase in the chain length of the alkyl group leads to an increase in the LCST of the polymer solutions [19]. In contrast, the ability of a given halide to lower the LCST decreases with its size. Since fluoride has the strongest tendency to form hydrogen bonds with water molecules, less water is available to solvate the macromolecules. These cations and anions follow the classical series demonstrated by Hofmeister for the salting out and salting in. According to von Hippel's analysis, this should effectively decrease the

number of solvent molecules available to solvate the polymer thereby making it easier for molecules to aggregate or salt out of solution [17].

2. The polymers studied

2.1 Introduction

Polymeric materials that respond to external stimuli such as temperature, pH, solvent quality, or light by changing their physical or chemical properties have been a topic of major interest in the last few decades [20, 21, 22]. Among them, temperature-responsive polymers were studied in great detail due to their possible applications in drug delivery systems (DDS) [23, 24], enzyme activity control [25, 26], solute separation through membrane [27], immunodiagnostic assays, purification and partitioning in biotechnology [23, 25]. However, their applications to DDS have been difficult because most of the thermosensitive polymers are nonbiodegradable and some are even toxic. Several polyacrylamides such as poly(N-isopropylacrylamide) (PNIPAM) and their copolymers that respond to environmental changes, as a rule, they are not biocompatible. Poly(N-isopropylacrylamide) (PNIPAM) is a thermosensitive polymer that exhibits phase separation in aqueous solution around 31^0C [2]. Its suitability as drug carrier is questioned by many because vinyl polymers are difficult to degrade in the body. In contrast, water-soluble polymers like poly(2-substituted-oxazoline) with properties such as biocompatibility, functionality, nontoxicity, and sterilizability are potential biomedical materials [28, 29]. From this point of view new functional materials are needed and consequently the temperature induced phase separation of these polymers aqueous solutions has attracted the

attention of many research groups [30, 31, 32]. This interest stems from the potential applications in various devices, in biomaterials and in drug delivery systems. For example poly(2-ethyl-2-oxazolines)(PEOZ), a water soluble polymer, was shown to have a lower toxicity than poly(ethylene glycol) [31]. Polymer complexes composed of PEOZ and poly(methacrylic acid) have been designed for uses in on-off insulin-released matrices [32]. PEOZ-based hydrogels also show swelling/deswelling behaviours in response to temperature and pH changes [33]. Amphiphilic block copolymers based on hydrophilic PEOZ and hydrophobic poly(*L*-lactide)(PLLA) have been designed as intelligent drug carriers [34]. Therefore, the synthesis and the study of novel poly(oxazolines) that possess a combination of these enabling properties is important, as it will help to develop new drug delivery systems thereby extending their field of applications. This thesis describes a study of two thermosensitive polymers, poly(2-isopropyl-2-oxazoline) and poly(N-vinylisobutylacrylamide), which are similar in structure to PNIPAM (shown in figure 3) but exhibit different properties in solutions.

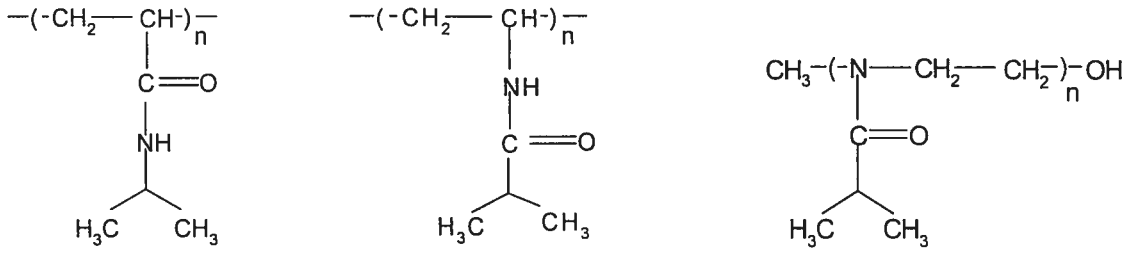

Figure 3 : Chemical structure of the repeat units of poly(N-isopropylacrylamide) (PNIPAM), poly(N-vinylisobutyramide) (PNVIBA), and poly(2-isopropyl-2-oxazoline) (PIPOZ).

2.2 Poly(2-isopropyl-2-oxazoline) (PIPOZ)

Poly(2-isopropyl-2-oxazoline) (PIPOZ) is prepared by cationic ring-opening polymerization of isopropyl oxazoline using a tosylate derivative as initiator, followed by termination with a NaOH/methanol solution yielding hydroxyl-terminated chains [35, 36]. Interestingly, PIPOZ aqueous solutions are temperature sensitive [37]: PIPOZ is soluble in cold water but separates from solution above a critical temperature (45-63^{0}C).

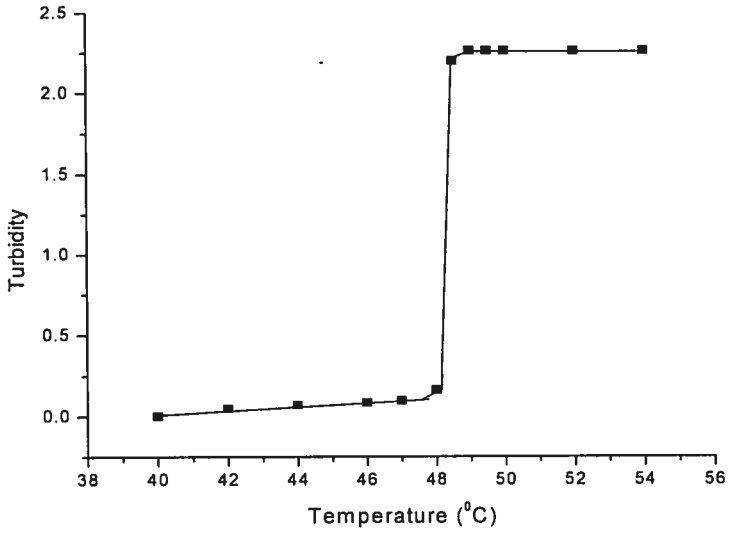

Figure 4 : Temperature dependence of the turbidity of $PIPOZ_{50}$-OH in H_2O . Polymer concentration: 1 g L^{-1}.

The cloud point of the solution of PIPOZ depends on molecular weight, the solvent (H_2O or D_2O), the concentration of the solution, and added salts.

As PIPOZ can be prepared by living polymerization, it can be obtained with low polydispersity and functional groups can be introduced at each chain end, thus enabling further modification [35, 36]. More importantly, it has been shown to be non cytotoxic and biocompatible [38]. These properties render PIPOZ derivatives useful as drug carriers, and as coatings of medical devices to improve their surface biocompatibility.

2.3 Poly(N-vinylisobutyramide) (PNVIBA)

Poly(N-vinylisobutyramide) has been prepared by free radical polymerization of N-vinylisobutyramide in a variety of solvent and initiators at 60^0C. The initiators used are potassium persulfate (KPS), 2,2-azobis(N,N-dimethyleneisobutylamidine) dihydrochloride (VA-044), benzoylperoxide (BPO), 2,2'-azobisisobutyronitrile (AIBN).

Poly(N-vinylisobutyramide) (PNVIBA) is a thermoresponsive, water-soluble polymer. Its solubility responds sharply to temperature changes (Figure 5).

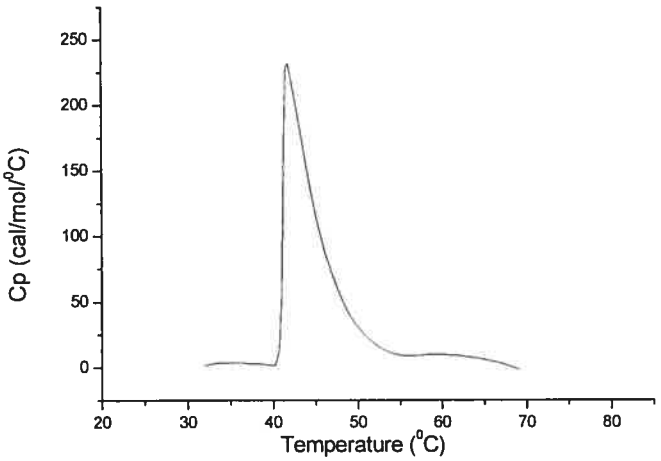

Figure 5 : Microcalorimetric endotherm for a solution of $PNVIBA_{82}$ in H_2O. Polymer concentration 1 g L^{-1}. Heating rate: 60 ^{0}C h^{-1}.

It shows an LCST that depends on the molecular weight of the polymer, the concentration of the solution, and the amount of added salt [39], this LCST can be increased or decreased by copolymerisation with hydrophilic or hydrophobic monomers [40]. Since it is possible to produce copolymers with wide variety of functions, PNVIBA derivatives are starting materials for various water soluble functional polymers [41]. Hydrogels based on PNVIBA with thermal and pH responsiveness have been prepared

using suitable cross-linkers [42, 43]. The solution properties of PNVIBA show many similarities to those of PNIPAM, but also exhibit subtle in several features of the phase transition process, such as transition sharpness, higher transition temperature for PNVIBA.

3. The microcalorimetry techniques:

3.1 Introduction

Most physico-chemical processes have an associated heat effect, therefore microcalorimetry can be used both for analytical applications and for fundamental studies of thermodynamic principles. The thermodynamic parameters that control macromolecular interactions in solution can be measured directly using microcalorimetric methods, and a large set of data is now available for protein folding, protein-ligand and protein-protein interactions [44]. Examples will be given here of the use of high sensitivity differential scanning calorimetry (DSC) [45, 46, 47], and the recently introduced technique of pressure perturbation calorimetry (PPC) [46, 47, 48, 49] which gives new information about hydration changes in temperature dependent molecular processes.

3.2 Differential scanning calorimetry (DSC):

3.2.1 The experiment

A differential scanning calorimeter measures the heat absorption or release in a sample as a function of temperature. A pair of matched cells is placed in a thermostated chamber. The sample cell is filled with a polymer solution. The reference cell is filled with an identical volume of solvent. The two cells are heated with a constant power input to their heaters during a scan. Any temperature difference

between the two cells is monitored with a feedback system so as to increase (or decrease) the sample cell power input. Since the masses and volumes of the two cells are matched, the power added or subtracted by the cell feedback system is a direct measure of the difference between the heat capacity of the sample and reference solutions. The cell feedback power is the raw data, usually expressed in units of cal/min, generated by a microcalorimetry scan. By knowing the scan rate (typically 1 ^{0}C/min) and the sample concentration, the output is converted into heat capacity (cal.mol^{-1}.^{0}C)) (Shown in figure 4) using the formula:

$$\frac{dQ_p}{dt}\frac{1}{\sigma M} = C_p \qquad (1)$$

where Q_p is the heat absorbed at constant pressure; t is the time; σ is the scan rate dT/dt; T is the temperature; M is the number of moles of sample in the sample cell and time is converted to temperature using the formula t.σ.

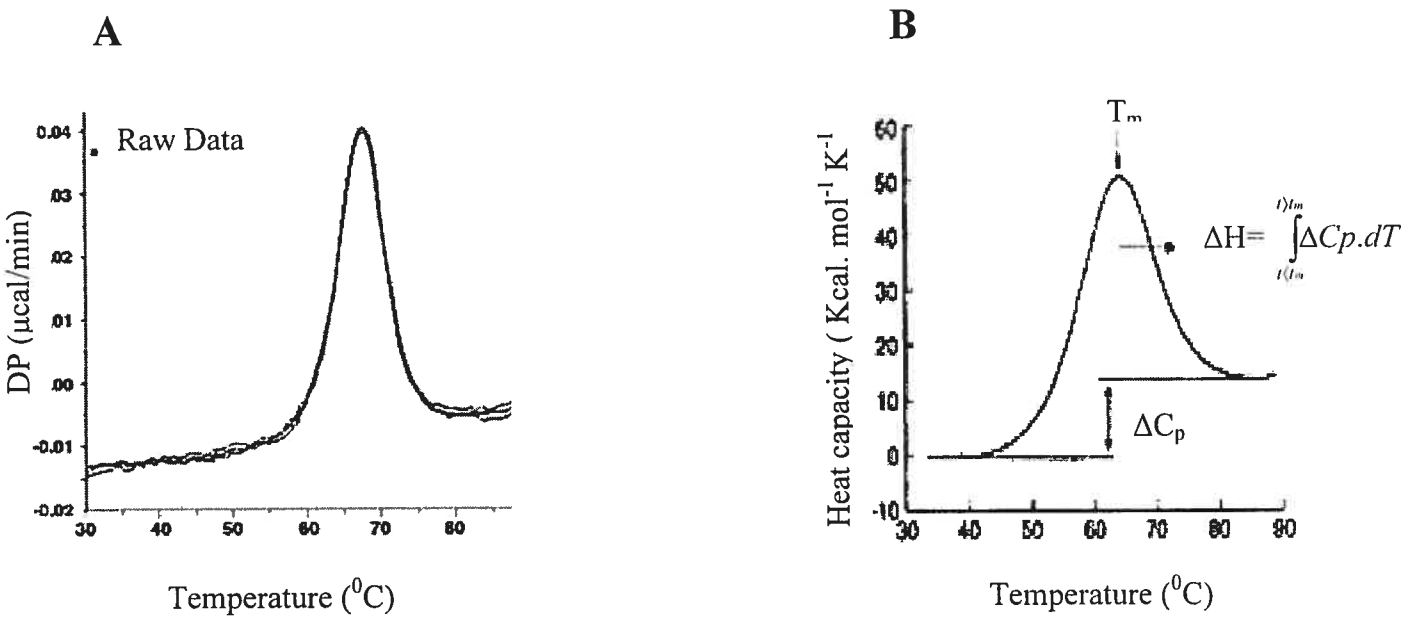

Figure 6 : (A) Raw data expressed in cal/min generated from a microcalorimetry scan. (B) the output converted into heat capacity (cal mol^{-1} ^{0}C).

3.2.2 Information obtained from DSC measurements

Differential scanning calorimetry (DSC) is used to obtain the thermodynamic parameters associated with heat-induced macromolecular transitions. The apparent molar heat capacity of a macromolecule in solution is measured as a function of temperature, subsequently yielding: the partial heat capacity, including changes in the enthalpy (ΔH), entropy (ΔS), heat capacity (ΔC_p) during a transition, and the temperature of the transition T_m. Simply, the temperature dependencies of ΔH and ΔS are the consequences of the significant heat capacity change ΔC_p associated with such interactions, and the relationship is given by the equations below:

$$\Delta H = \int_{T<T_m}^{T>T_m} \Delta Cp.dT = \Delta C_p(T_{T>Tm} - T_{T<Tm}) \quad (2)$$

$$\Delta S = \int_{T<T_m}^{T>T_m} \frac{\Delta C_p}{T} dT = \Delta C_p \ln (T_{T>Tm} / T_{T<Tm}) \quad (3)$$

where $T>T_m$ *is the temperature range above the phase transition temperature and* $T<T_m$ *is the temperature range below the phase transition temperature.*

3.2.3 Domains of application

DSC techniques have been used to evaluate the thermal stability of solute and to detect phase transitions associated with macromolecules in solutions. It has become a widely used tool for studying conformational transitions of proteins, lipid assemblies and polymers [50, 51]. In the last decade, the applications of this technique have grown rapidly due the availability of highly sensitives instrument which are easy to use, and yield highly reproducible results.

3.3 Pressure perturbation calorimetry (PPC):

3.3.1 Introduction

Pressure perturbation calorimetry is a technique that measures the heat change in a solution that results when the pressure above the solution is changed. When used in a differential calorimeter containing a dilute solution of solute in the sample cell and the solvent in the reference cell, the differential heat measured can be used to calculate the coefficient of thermal expansion of the solute α, $\alpha = 1/V(\partial V/\partial T)_P$. For proteins and polymers in dilute aqueous solution, α_{macr} is dominated by a temperature-dependent contribution arising from the interaction of the macromolecule with water functional groups. This arises due to the effect of macromolecule groups on the hydrogen-bonded structure of water molecules surrounding the polymer chain, and thereby clearly differentiates between structure-making hydrophobic groups and structure-breaking hydrophilic groups. The technique can be extended to calculate the changes in the volume of the solvation layer around a polymer chain before and after a phase transition.

3.3.2 The experiment

The PPC measurement can be performed in a DSC instrument fitted with a special accessory that applies a pressure increase followed by pressure release to a sample kept at constant temperature. The maximum pressure applied is 500 kPa. The calorimetric baseline is equilibrated at constant temperature and pressure P_1. The pressure is then changed to P_2 causing heat to be absorbed in both cells. The compensation heat (ΔQ_{rev}) required to maintain the sample temperature constant due to pressure changes is just the reverse of the thermal response, dQ/dP at constant

temperature and is obtained by integrating the area under each peak. The temperature is then changed, the baseline is allowed to equilibrate and the compression /decompression is repeated. A number of such pressure jumps are performed automatically at different temperatures yielding the expansivity as a function of temperature, $\alpha = 1/V(\partial V/\partial T)_p$ using the relationship:

$$\frac{dQ}{dP} = T\frac{dV}{dT} \qquad (4)$$

and taking into account blank data for solvent (sample cell) versus water (reference cell), solvent versus solvent, and water versus water. The equations involved in the PPC data analysis are presented in details in appendix A. The PPC experimental setup and the experimental PPC trace registered for a solution of polymer [2] are shown in figure 5 and figure 6.

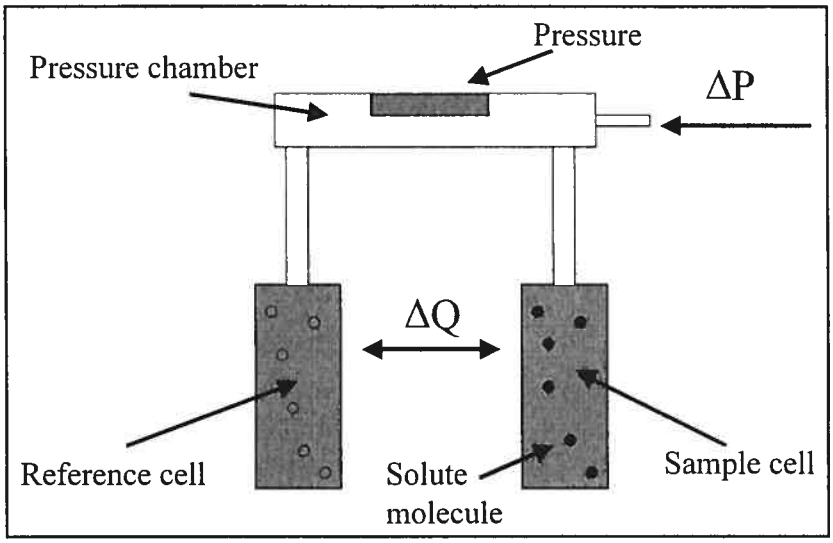

Figure 7 : Schematic diagram of the pressure perturbation calorimetry (PPC) experimental setup. The open circles in the reference cell represent the volume occupied by solvent in this cell, which counterbalances the volume occupied by the solute molecules in the sample cell.

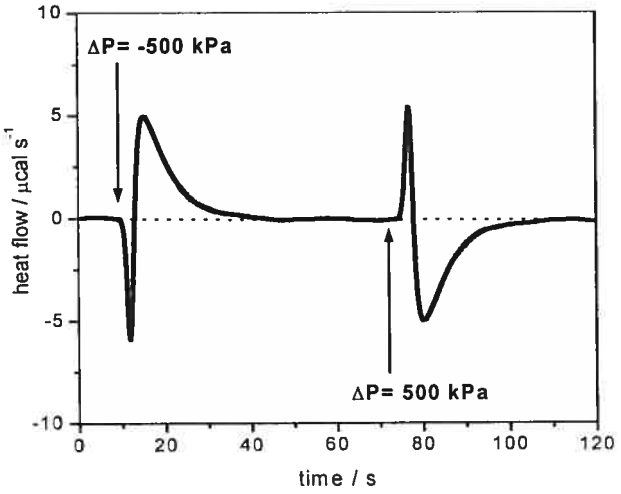

Figure 8 : Experimental PPC trace registered for a solution poly(N-isopropylacrylamide) in H_2O. Polymer concentration: 5g L^{-1}, temperature = 20 ^{0}C.

3.3.3 Information obtained from PPC measurements

The data obtained from consecutive pressure increase and decrease cycles at the same temperature are averaged, to construct for the PPC curve. The area under the curve gives the volume change experienced by a solute in a given temperature range accompanying the transition. In the case of a phase transition, the PPC experiment measures the thermal expansion coefficient α_{macr}, the phase transition temperature T_m, and the effect of pressure on this phase transition temperature.

3.3.4 Domains of application

PPC is able to measure extremely small temperature changes (0.05 ^{0}C) induced by weak pressure jumps. It has been used to study protein folding and unfolding [52],

it also offers interesting perspectives in the study of lipids and membranes and allows the characterization of the kinetics of the lipid gel-to-liquid crystal transition [53]. Kujawa and Winnik [2] have demonstrated that PPC can be used to measure the thermal expansion coefficient, α_v, of thermosensitive polymers as function of the temperature, the effect of the pressure on the phase transition temperature T_m, and the volume changes, ΔV, associated with different types of polymers phase transitions.

4. The scope of the thesis:

The main objective of this thesis is to study of the solution properties of two new water-soluble thermosensitive polymers using high sensitivity differential scanning calorimetry, pressure perturbation calorimetry, and turbidity measurements. These techniques yield the thermodynamic parameter controlling the heat-induced phase transitions and their influence on the solubility of these materials.

Chapter two pertains to the study of the temperature-induced phase separation of aqueous solutions of poly(2-isopropyl-2-oxazolines) obtained by cationic ring opening polymerization. The synthesis and the physicochemical properties of the polymers are reported.

In chapter three, a micocalorimetric study of poly(N-vinylisobutyramide) (PNVIBA) [54, 55, 56] is presented. Effects of polymer concentration, polymer molecular weight, and concentration of added salts are examined. Also, the properties of PNVIBA aqueous solutions are compared to those of PNIPAM.

Finally, chapter four is a conclusion, which summarizes my study in this thesis, also, some examples of future applications of the polymers studied in this work will be shown and discussed.

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CHAPTER 2

Presentation of the article

Microcalorimetric Study of the Temperature-Induced Phase Separation in Aqueous Solutions of Poly(2-isopropyl-2-oxazolines)

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1. Abstract

The effect of temperature on aqueous solutions of poly(2-isopropyl-2-oxazoline) (PIPOZ) samples of molecular weights ranging from 1,900 to 5,700 g mol^{-1} was monitored by turbidimetry, high sensitivity microcalorimetry (HS DSC), and pressure perturbation calorimetry (PPC) from 10 °C to 80 °C. The polymers were soluble in cold water and underwent phase separation at $T_{Cp} \sim 45$ - 63°C, depending on their molecular weight. The phase transition was endothermic, with an enthalpy change ranging from 0.36 ± 0.01 to 1.16 ± 0.01 kcal mol^{-1}. The coefficient of thermal expansion of PIPOZ in water (α_{pol}), determined by PPC, underwent a sharp increase at the temperature corresponding to the onset of phase transition, reaching a maximum value at $T \sim T_{Cp}$. Microcalorimetric measurements were carried out with solutions of PIPOZ samples in D_2O and in aqueous NaCl solutions. The thermodynamic and volumetric changes associated with the phase transition of aqueous PIPOZ solutions are compared to those of aqueous solutions of two related polymers, poly(vinylcaprolactam) and poly(*N*-isopropylacrylamide) (PNIPAM), a polymer structurally related to PIPOZ that undergoes a phase transition in water at $\sim 31^0$C.

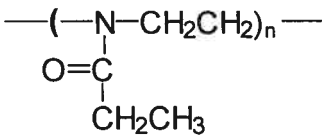
2. Introduction

A large body of work has been devoted over the past decades toward the development of drug delivery systems based on polymeric micelles featuring longevity in blood circulation.[1-6]. In most studies, these drug vehicles consist of a hydrophilic poly(ethylene glycol) (PEG) outer corona, since this polymer prevents non-specific adsorption of the polymeric micelle to proteins and cells, thus allowing the micelles to evade recognition by the reticuloendothelial system.[3, 7, 8] There has been little investigation into the use of hydrophilic corona forming polymers other than PEG to create micellar drug delivery systems. Charged hydrophilic polymers have been used for delivery to mucosal surfaces, such as the respiratory, gastrointestinal, and urogenitary tracts.[9] Polymeric micelles with a corona made up of natural or semi-synthetic polysaccharides are investigated as well as vehicles in the oral administration of poorly-water soluble drugs.[10, 11] Recently, polymeric micelles based on block copolymers of 2-ethyl-2-oxazoline (PEOZ) and ε-caprolactone [12] were evaluated as drug carriers and shown to have low cytotoxicity and hemolytic activity.[13]

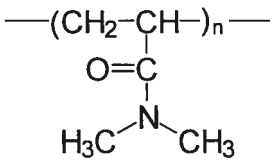
In contrast to PEG or polysaccharide systems, poly(2-alkyl-2-oxazolines), synthesized by living cationic polymerization, can be tailored through the choice of not only the end groups but also the side chains.[14-16] The length of the alkyl substituents controls to a large extent the relative hydrophilicity of the poly(2-alkyl-2-oxazolines), and only few oxazolines, such as the 2-methyl-, 2-ethyl-, and 2-isopropyl-2-oxazolines, lead to polymers soluble in water at room temperature.[17] The biocompatibility and interfacial characteristics of poly(2-methyl-2-oxazolines) (PMOZ) and PEOZ have been assessed in various biotechnological applications.[18-20] Lipid vesicles doped with poly(2-ethyl-2-

oxazoline) lipopolymers exhibited enhanced blood circulation times compared to ordinary phospholipid vesicles.[21] The ability of the PMOZ and PEOZ chains to elicit beneficial properties when grafted onto the surface of liposomes has been explained by the conformational mobility of the polymers and the tendency of their repeat units to form hydrogen bonds with water.[21] However, quantitative information on the interactions of poly(2-alkyl-2-oxazolines) with water is scarce compared to the wealth of data dealing with the behavior of PEG in water.[22]

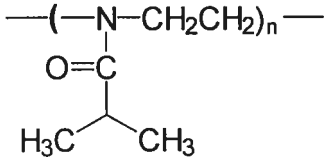
Among the various poly(2-alkyl-2-oxazolines), PEOZ has attracted the most attention, by far. Aqueous solutions of PEOZ exhibit a cloud point around 62-65 °C depending on molecular weight and concentration.[23] The cloud point temperature is affected by the presence of cosolvents, such as dioxane, and of salts, decreasing in the presence of sodium chloride, but increasing upon addition of tetrabutylammonium bromide.[24] Studies of PMOZ and PEOZ in water by light scattering revealed that their second virial coefficients decrease with increasing solution temperature, an indication of the involvement of water/polymer hydrogen bonds in the mechanism of temperature-induced phase separation.[25, 26] The phase transition of aqueous PEOZ solutions was investigated over the entire water/polymer composition domain. Concentrated PEOZ solutions obey the "classical" Flory-Huggins miscibility behavior: the cloud point shifts to lower polymer concentration as the molecular weight of the polymer increases (M_{vis} 22,000 - 170,000 g mol^{-1}).[25, 27] As pointed out by Lin *et al.*,[24] the repeat unit of PEOZ is isomeric to that of poly(*N, N*-dimethylacrylamide) (Figure 1), and, as such, there are similarities in the solubility and miscibility characteristics of the two polymers; yet, there are also important differences. Although both polymers are soluble in water, only PEOZ



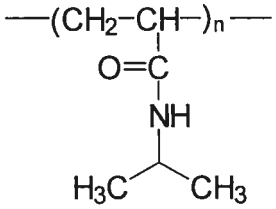
Poly(2-ethyl-2-oxazoline)



Poly(N,N-dimethylacrylamide)



Poly(2-isopropyl-2-oxazoline)



Poly(N-isopropylacrylamide)

Figure 1 : Chemical structure of the repeat units of poly(2-ethyl-2-oxazoline) (PEOZ), poly(N,N-dimethylacrylamide), poly(2-isopropyl-2-oxazoline) (PIPOZ), and poly(N-isopropylacrylamide) (PNIPAM).

aqueous solutions exhibit a cloud point below 100 °C, when the polymer molecular weight exceeds a threshold value of ~ 10,000 g mol^{-1}.[27] In this respect, one may note that the repeat unit of poly(2-isopropyl-2-oxazoline) (PIPOZ) is isomeric to that of poly(*N*-isopropylacrylamide) (PNIPAM) (Figure 1), a water-soluble polymer that exhibits a cloud point in water at 31 °C.[28] Indeed, PIPOZ in water presents a cloud point, as revealed by turbidity studies.[29] The cloud point of a solution of PIPOZ (M_n 16,700) ranges from 39 °C to 36 °C, depending on polymer concentration. Addition of NaCl depresses the solution cloud point, while surfactants such as sodium dodecyl sulfate and *n*-dodecyltrimethylammonium chloride have the opposite effect. From these data, one

detects similarities in the solution properties of PIPOZ and PNIPAM. Nonetheless, cloud point values give little indication on the differences in the state of hydration of the two polymers.

As part of a project aimed at the development of novel PIPOZ-based thermoresponsive polymeric micelles, we prepared recently a series of PIPOZ oligomers terminated with a hydroxyl group. To understand the solution properties of the ultimate polymeric micelles, we set about to study the properties of aqueous solutions of PIPOZ as they undergo phase transition. Two techniques, high-sensitivity differential scanning calorimetry (DSC) and pressure perturbation calorimetry (PPC) were employed. From the DSC measurements, we retrieve the temperature and the enthalpy of the phase transition of solutions of oligomers of various molecular weights and polydispersities. From the PPC results, we derive the volumetric properties of PIPOZ solutions as they undergo a phase transition. Pressure perturbation calorimetry measures the heat absorbed or released by a solution due to a sudden pressure change at constant temperature.[30] The heat can be used to calculate the coefficient of thermal expansion of the solute and its temperature dependence. The technique has been exploited to obtain the changes in the volume of the solvation layer around proteins before and after unfolding,[31] to monitor the volume changes accompanying the pretransition and transition of phospholipid bilayers,[32] and to evaluate the volume changes of the solvation layer during the coil/globule collapse in water of PNIPAM [33,34], hydrophobically-modified PNIPAM samples,[33,34] and poly(vinylcaprolactam).[35]

Solutions of PIPOZ in H_2O, D_2O, and aqueous NaCl solutions were examined by the two calorimetry techniques, in order to assess the relative importance of

water/polymer hydrogen bond formation and polymer/polymer hydrophobic interactions. Light and heavy water are chemically identical, yet their physical properties differ significantly.[36] The dissimilarities between H_2O and D_2O are believed to stem from differences in the intermolecular hydrogen bonds energies. The lengths of hydrogen bonds in the two liquids are about the same, but a "hydrogen" bond in D_2O is about 5 % stronger than a hydrogen bond in H_2O. Overall, D_2O is a more "structured" solvent than light water.[37]

In a first section, we present the preparation of the oligomers and describe their chemical characteristics obtained from ^1H NMR spectroscopy, MALDI-TOF mass spectrometry, and gel permeation chromatography (GPC). Then, we report the results of the microcalorimetry study of PIPOZ samples, placing particular emphasis on the effect of the polymer chain length on its behavior during the solution phase transition. Taken together, the results suggest a description of the PIPOZ/water interactions, which will be compared to the currently accepted view of the phenomena taking place in aqueous solutions of PNIPAM, the chemical isomer of PIPOZ.

3. Experimental Section:

3.1 Materials:

Water was deionized with a Millipore Milli-Q water purification system. Isobutyric acid, 2-aminoethanol, and methyl *p*-tosylate were purchased from Wako Pure Chemical Industries. Deuterium oxide (99.8 %) was purchased from Aldrich-Sigma Chemicals. PNIPAM was prepared by RAFT polymerization, using a trithiocarbonate RAFT agent.[38]

3.2 Instrumentation:

NMR spectra were recorded on a Bruker ARX-400 400 MHz spectrometer. UV/Visible spectra were measured with a Hewlett Packard 8452A photodiode array spectrometer equipped with a Hewlett Packard 89090 temperature controller. Gel permeation chromatography (GPC) measurements were performed with a TOSOH HLC-8220 system equipped with a TOSOH differential refractometer, two TOSOH Super HHZ columns (4000 and 3000) eluted with DMF containing triethylamine (30mM) and LiCl (10 mM) (flow rate 0.8 mL min^{-1}, 40 °C) and calibrated with poly(ethyleneglycol) standards used to determine the molecular weights. MALDI-TOF mass spectra were recorded with a Reflex III (Bruker). All spectra were obtained in positive ion mode and ionization was performed with a 337 pulsed (3ns pulse width, 10^6 to 10^7 W cm^{-2}) nitrogen laser. An external calibration was performed using poly(ethylene glycol) (MeO-PEG-OH) of Mw 5,000 (NOF Corporation). To prepare the matrix, a solution of the polymer in THF (20 µL, 6 mg mL^{-1}) was mixed with a solution of 8,9-trihydroxyanthracene in THF (20 µL, 10 mg mL^{-1}) by sonication for a few minutes. A solution of lithium trifluoroacetate in THF (2 µL, 2 mg mL^{-1}) was added to the matrix. The resulting mixture was shaken briefly and applied to the probe (2 µL)

4 Synthesis and measurements:

4.1 Preparation of 2-Isopropyl-2-oxazoline.[39]

Isobutyric acid (132g, 1.5 mol) was added to 2-aminoethanol (92 g, 1.5 mol) under stirring. The resulting salt was heated slowly to 230 °C, a temperature higher than its melting point. The liquid was refluxed for 48 h, followed by azeotropic distillation at 110

°C. The distillate was diluted in dichloromethane (350 mL) to separate water from 2-isopropyl-2-oxazoline. The aqueous layer was extracted repeatedly with dichloromethane. The combined organic layers were concentrated in vacuo. The residue was purified by repeated distillations in the presence of CaH_2, yielding dry 2-isopropyl-2-oxazoline (72 g, b.p. 138 °C). ^{1}H NMR ($CDCl_3$, δ) 1.11 (d, 6 H, $(CH_3)_2CH$-), 2.49 (m, 1 H, $(CH_3)_2CH$-), 3.79 (t, 2 H, NCH_2CH_2O), 4.18 ppm (t, 2H, NCH_2CH_2O).

4.2 Polymerizations:

Hydroxy-Terminated Poly(2-isopropyl-2-oxazolines)

A solution of 2-isopropyl-2-oxazoline (9.74 g, 86 mmol) and methyl *p*-tosylate (0.30 g, 1,0 mmol) in nitromethane (30 mL) was stirred at 40 °C under argon. Aliquots of the polymerization solution (1.0 mL) were taken at various time intervals (48 , 72, 120 , and 168 hr). They were cooled to room temperature and poured into a mixture of aqueous NaOH (1.5 mL, 1.0 M) and methanol (1.5 mL). The resulting solution was dialyzed against water for 2 days. The polymer was isolated by freeze-drying. ^{1}H NMR ($CDCl_3$, δ) 1.1 (br s, $[(CH_3)_2CH$-], 2.66 and 2.89 (br m, $[(CH_3)_2CH$-], 3.08 (s, terminal CH_3), 3.54 (br s, $-(CH_2-CH_2-N)-$).

4.3- Cloud point determinations:

Cloud points were determined by spectrophotometric detection of the changes in turbidity (λ = 600 nm) of aqueous polymer solutions (1.0 g L^{-1}) heated at a constant rate (1 °C min^{-1}) in a magnetically stirred UV cell. The value reported is the temperature corresponding to a decrease of 20 % of the solution transmittance.

4.4 Differential scanning calorimetry (DSC):

DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc.) at an external pressure of ca. 180 kPa. The cell volume was 0.52 mL. The heating rate was 1.0 °C min^{-1}, and the instrument response time was set at 5.6 s. Data were corrected for instrument response time and analyzed using the software supplied by the manufacturer. The polymer concentration ranged from 0.5 to 2.0 g L^{-1}.

4.5 Pressure perturbation calorimetry (PPC):

PPC measurements were performed on a VP-DSC microcalorimeter equipped with a pressure perturbation accessory (MicroCal Inc.). The pressure applied during the compression cycle was 500 kPa. The reference cell and sample cell volumes were identical (0.52 mL). The polymer concentration was 5 .0 g L^{-1}. Control experiments were performed, namely water (sample cell) *vs* water (reference cell), H_2O *vs* D_2O, D_2O *vs* D_2O, NaCl in water *vs* NaCl in water, and water *vs* NaCl in water. The PPC accessory has been described in detail elsewhere.[30] Briefly, it applies a pressure of 500 kPa, then a pressure release to ambient pressure, to the sample, which is kept at constant temperature. The temperature of the sample cell is kept constant by compensation of the heat change caused by the pressure jump. After equilibration, an upward pressure jump (500 kPa) is performed. The heat peaks caused by the compression and decompression are opposite in sign but agree in absolute value. A large number of compression/decompression cycles are performed at each temperature.

The coefficient of thermal expansion of a polymer solute in a solvent is obtained from Equation 1[33],

$$\alpha_{pol} = \alpha_{solv} - \frac{\Delta Q_{rev}}{T\Delta P m_{pol} \overline{V}_{pol}} \qquad (1)$$

where α_{pol} and α_{solv} are the thermal expansion coefficients of the polymer and the solvent, respectively, ΔQ_{rev} corresponds to the heat consumed or released upon the small pressure changes, ΔP is the change in pressure, T is the temperature, $\overline{V}_{pol}$ is the partial specific volume of the polymer, and m_{pol} is the mass of polymer in solution. The partial specific volumes of the polymers were determined by an increment method based on the group contribution theory developed to estimate $\overline{V}$ of aqueous systems and estimated to be accurate within 2 %.[40] Thus, for PIPOZ, $\overline{V}$ = 0.888 $cm^3\ g^{-1}$ and for PNIPAM, $\overline{V}$ = 0.894 $cm^3\ g^{-1}$.

The change in volume of the solvation layer of the polymer (ΔV) during a phase transition is obtained by integration of the curve of the changes in the coefficient of thermal expansion with temperature (eq 2), assuming that ΔV is small compared to $\overline{V}$, the intrinsic volume of the polymer. A complete deviation of the equations has been reported previously.[30,33] Data were analyzed using the software supplied by the manufacturer.

$$\frac{\Delta V}{V} = \int \alpha dT \qquad (2)$$

5. Results:

5.1 Preparation and Characterization of the Polymers:

The oligomers were synthesized by living cationic polymerization, initiated with methyl *p*-tosylate, of 2-isopropyl-2-oxazoline, followed by termination with a NaOH/methanol solution yielding hydroxyl-terminated chains (Figure 2).

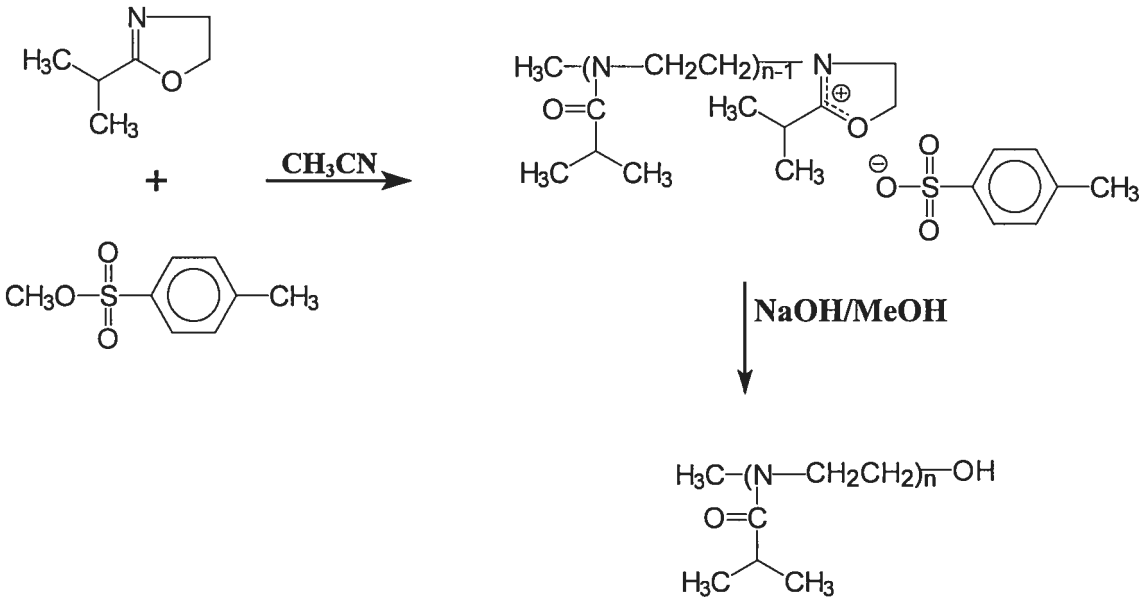


Figure 2 : Reaction scheme for the preparation of poly(2-isopropyl-2-oxazolines) and structure of the polymer.

Polymerization conditions were selected to achieve the narrow molecular weight polydispersity and low polymer molecular weight required by the ultimate application of the PIPOZ chains in drug delivery vehicles. One set of samples ($PIPOZ_n$-OH, n = 17, 21, 41, and 50; Table 1) was obtained by sampling a single polymerization mixture

at various time intervals. This method provided oligo(2-isopropyl-2-oxazolines) of narrow polydispersity index, as determined by GPC (Table 1) and MALDI-TOF mass spectrometry (Figure 3).

Polymer	$[M_o]/[I_0]$[a]	Time (h)	M_n[b] ($g\ mol^{-1}$)	M_w/M_n[b]	M_n[c] ($g\ mol^{-1}$)	DP[c]
$PIPOZ_{17}$-OH	86	48	1,700	1.05	1,900	17
$PIPOZ_{21}$-OH	86	72	2,400	1.04	2,400	21
$PIPOZ_{41}$-OH	86	120	4,300	1.03	4,600	41
$PIPOZ_{50}$-OH	86	168	5,700	1.03	5,650	50
PNIPAM	-----	----	13,000	1.16		

[a] initial monomer/initiator feed ratio
[b] from GPC measurements
[c] from ^1H NMR spectra in $CDCl_3$

Table 1 : Polymerization conditions and characteristics of the poly(2-isopropyl-2-oxazolines).

The mass spectrum of PIPOZ-OH (M_n 6,190) presented in Figure 3 is also dominated by one main ion population. The spacings of the $\Delta m/z$ signals, 113.192, are in good agreement with the expected mass of one monomer unit. The major population of signals was attributed to the Li^+ adduct and the minor population of signals to the Na^+ adduct. On the basis of the mass spectra, it can be assumed that all oligomers bear the

desired end-functionalities, namely a methyl group at one chain end and either a hydroxyl or a primary amine group at the other chain end.

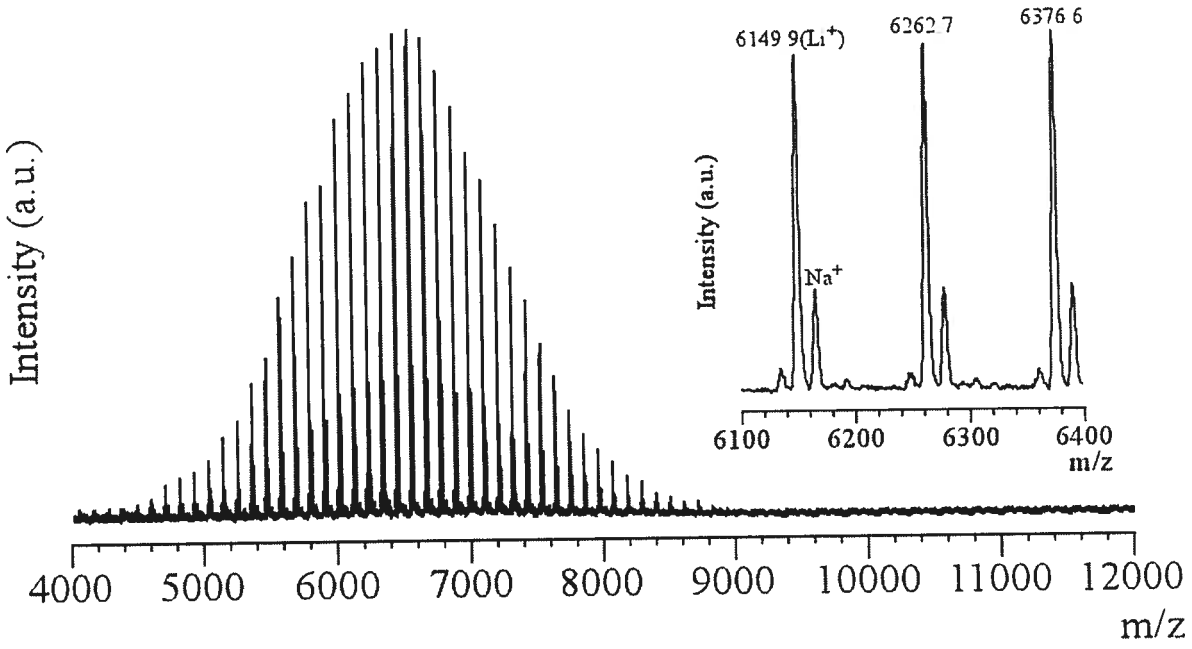


Figure 3 : MALDI-TOF mass spectrum of $PIPOZ_{50}$-OH obtained after 168 h polymerization. An expanded view of the spectrum ranging from 6100 to 6400 amu is given in the inset.

The degree of polymerization of the oligomers was calculated from the ^1H NMR spectra of the samples in chloroform-D, using the broad singlet at δ 1.11 ppm, ascribed to the resonance of the methyl protons of the isopropyl group of the repeat unit, together with the singlet at δ 3.08 ppm, attributed to the resonance of the terminal methyl protons (Figure 4). The number average molecular weights derived from integrations of these ^1H

NMR signals are in excellent agreement with the molecular weights determined by GPC (Table 1) and MALDI-TOF mass spectra (Figure 3).

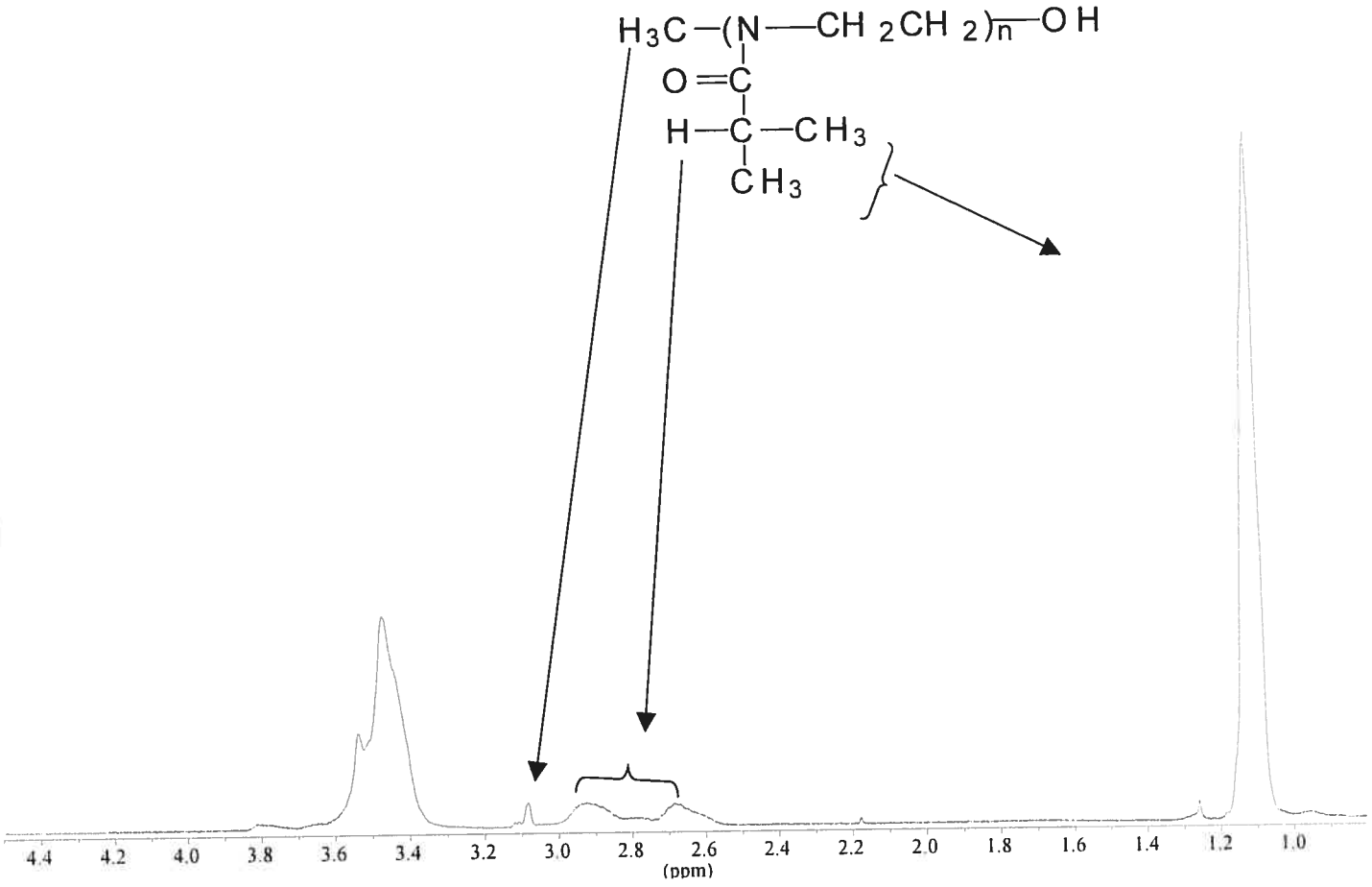


Figure 4 : ^{1}H NMR spectrum of $PIPOZ_{21}$-OH in $CDCl_3$.

5.2 Phase transition of the oligomers in water

5.2.1 Turbidity measurements:

The fastest method to determine the cloud point of a polymer solution consists of measuring the changes in turbidity as the solution is heated at a constant rate.

Polymer	Solvent	T_{CP} (°C)[a]	T_{onset} (°C)[b] (DSC)	T_{max} (°C)[b] (DSC)	ΔH (kcal mol^{-1})[b] (DSC)	T_{max} (°C)[c] (PPC)	ΔV/V (%)[c]
$PIPOZ_{17}$-OH	H_2O	72.5 ± 0.2	73.1 ± 0.2	76.5 ± 0.3	0.36 ± 0.01	65.0 ± 0.3	0.43
	D_2O		68.9 ± 0.2	75.2 ± 0.3	0.39 ± 0.01	63.0 ± 0.3	0.70
$PIPOZ_{21}$-OH	H_2O	62.8 ± 0.2	61.4 ± 0.2	64.0 ± 0.3	0.72 ± 0.01	52.5 ± 0.3	0.64
	D_2O	60.3 ± 0.2	58.9 ± 0.2	62.1 ± 0.3	0.85 ± 0.01	53.0 ± 0.3	1.00
$PIPOZ_{41}$-OH	H_2O	51.3 ± 0.2	51.3 ± 0.2	54.0 ± 0.3	0.90 ± 0.01	45.2 ± 0.3	0.84
	D_2O		45.2 ± 0.2	51.3 ± 0.3	1.16 ± 0.01	47.5 ± 0.3	1.30
$PIPOZ_{50}$-OH	H_2O	48.1 ± 0.2	46.5 ± 0.2	48.0 ± 0.3	1.35 ± 0.01	45.0 ± 0.3	1.10
	D_2O		46.9 ± 0.2	47.0 ± 0.3	1.43 ± 0.01	43.0 ± 0.3	1.80
	NaCl (0.5 M)		37.1 ± 0.2	40.6 ± 0.3	1.61 ± 0.01	37.0 ± 0.3	1.30
	NaCl (1.0 M)		33.5 ± 0.2	35.0 ± 0.3	1.76 ± 0.01	32.0 ± 0.3	1.40
PNIPAM	H_2O		24.5 ± 0.2	32.6 ± 0.3	0.80 ± 0.01	32.0 ± 0.3	0.90
	D_2O						
	NaCl (0.5 M)		18.4± 0.2	25.6 ± 0.3	1.20 ± 0.01	26.0 ± 0.3	1.00
	NaCl (1.0 M)		13.6 ± 0.2	20.1 ± 0.3	1.37 ± 0.01	21.0 ± 0.3	1.20
PNIPAM-COOH[d] (Mn ~ 2,700)	H_2O	32.7[d]		31.7[d]	0.45[d]		

[a] from turbidity measurements (polymer concentration: 1.0 g L^{-1})
[b] from DSC measurements (polymer concentration: 1.0 g L^{-1})
[c] from PPC measurements (polymer concentration: 5.0 g L^{-1})
[d] from reference 43

Table 2 : Thermodynamic parameters of the poly(2-isopropyl-oxazolines).

The temperature of turbidity onset, defined here as the cloud point temperature, T_{CP}, decreases with increasing PIPOZ molecular weight (Table 2). The turbidity increased sharply as the temperature exceeded this onset value. To ascertain the reversibility of the phase transition, polymer solutions heated to 70 °C were kept at this temperature for 30 min and then cooled to room temperature at constant cooling rate. The solutions became clear in all cases, but the temperature for which 80 % transmission was recovered was lower by ~ 2 °C, than T_{CP} recorded during a heating scan. The cloud point temperature of a solution of $PIPOZ_{21}$-OH (1.0 g L^{-1}) was determined in light and heavy water: it was lower by ~ 1.5 °C in D_2O compared to H_2O (Table 2). Note that this trend is in agreement with an observation made by Chen et al. in their study of a polydisperse sample of PEOZ (M_w 116,000). They reported that use of D_2O in place of H_2O yielded a cloud point some 3 °C lower.[25]

5.2.2 High sensitivity differential calorimetry:

The changes with temperature of the partial excess heat capacity C_p of aqueous solutions of several PIPOZ-OH samples (1 g L^{-1}) are presented in Figure 5. The thermograms are endothermic, broad, and markedly asymmetric, with a sharp increase in heat capacity on the low-temperature side (onset of the transition, T_{onset}) and a gradual decrease of the heat capacity for temperatures higher than a maximum temperature T_{max}. For all polymers the values of C_p were the same, within experimental error, before and after the transition. Neither changing the concentration of the solution, from 0.5 to 5 g L^{-1} nor varying the scanning rate, from 10 to 90 °C/h, affected the shape of the thermograms.

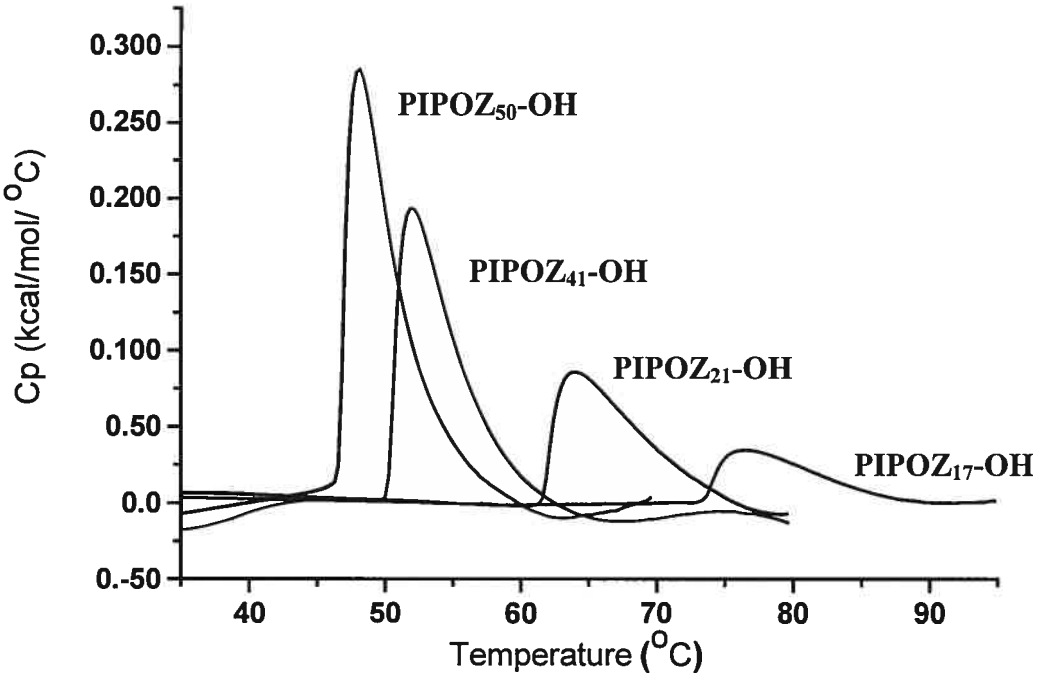


Figure 5 : Microcalorimetric endotherms for aqueous solutions of PIPOZ-OH samples in water. Polymer concentration: 1.0 g L^{-1}. Heating rate: 60 °C h^{-1}.

Therefore, the characteristic times of the transitions are shorter than those of the thermal equilibration of the cells. The reproducibility of the thermograms was demonstrated during the second and third heating at the same heating rate. The transition observed is completely reversible.

From plots of the partial heat capacity of PIPOZ solutions *vs* temperature, one can extract three thermodynamic parameters: T_{onset}, T_{max}, and ΔH, the enthalpy of the transition (Table 2). The values of T_{onset} and T_{max} follow the same trends as the values of T_{Cp}: for a given polymer, they decrease with increasing concentration, and, for solutions

of identical concentration, they decrease with increasing polymer molecular weight (Figure 6). The enthalpy of transition, as well, exhibits a significant dependence on molecular weight: it increases from 0.36 ± 0.01 kcal (mol of monomer unit)$^{-1}$ for the shortest oligomer to 1.40 ± 0.01 kcal mol^{-1} for the longest (Figure 6). The latter value corresponds approximately to the energy required to break one hydrogen bond per repeat unit.[41] It would appear, therefore, that the availability of the carbonyl groups in the PIPOZ chain to form hydrogen bonds with water increases with molecular weight. A similar observation was made by Chen *et al.*[26] in their light scattering studies of PEOZ in water: they report that, for solutions of this polymer in water, the excess enthalpy of dilution becomes more negative with increasing polymer molecular weight.

The phase transition of PIPOZ oligomers was affected by the presence of NaCl: increased electrolyte concentration decreased the phase transition temperature and increased the transition enthalpy (Table 2). Both phenomena are consistent with the well-known 'salting-out' effect of NaCl and corroborate observations of Uyama and Kobayashi in their study of a PIPOZ sample of higher molecular weight,[29] and of Lin et al. in their investigation of PEOZ.[24]

Thermograms recorded for polymer solutions in D_2O exhibit the same skewed shape as the thermograms of polymer solutions in H_2O, as exemplified in Figure 6 (top), where we show DSC scans for solutions of PIOPZ$_{50}$-OH in heavy and light water. In all cases, the T_{onset} and T_{max} values recorded for solutions in D_2O are lower than those obtained for solutions in H_2O, and the differences between solutions in the two solvents increase as the molecular weight of the polymer decreases.

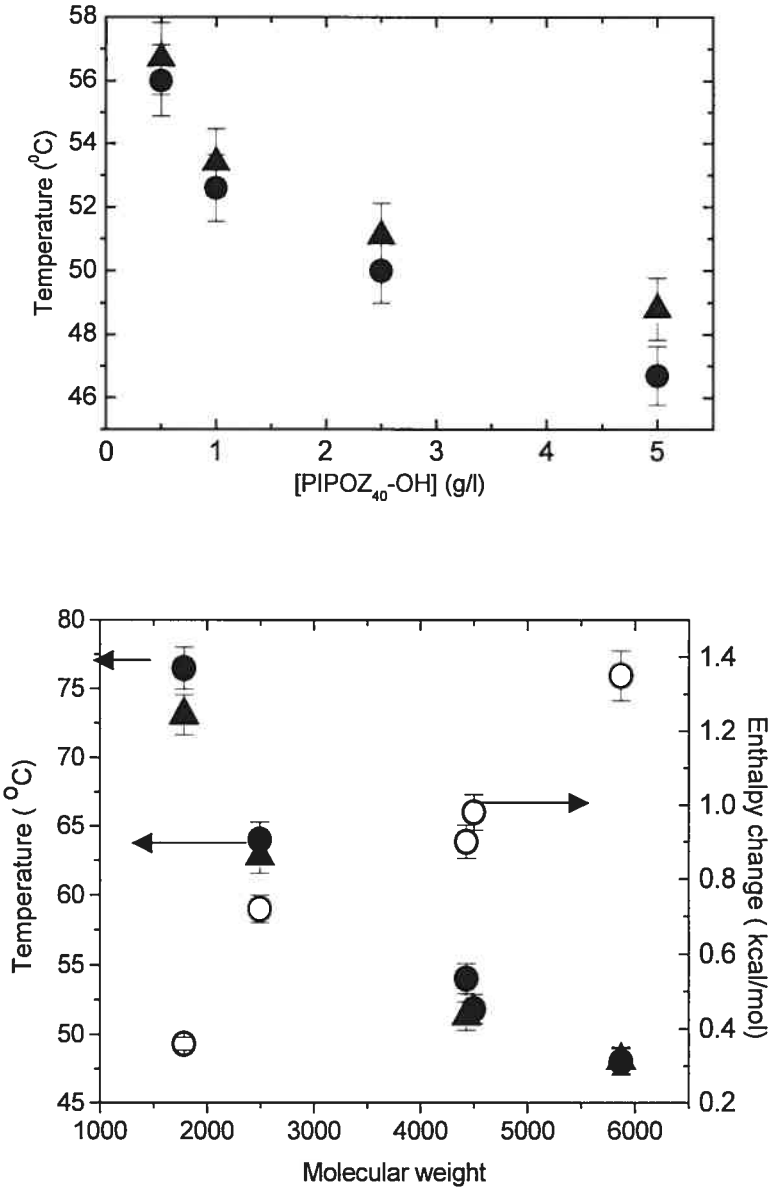


Figure 6 : (top) Plots of the changes as a function of polymer concentration of the transition temperature, T_m (full circle), and of the cloud point, CP (full triangle), for aqueous solutions of $PIPOZ_{41}$-OH. (bottom) Plots of the changes as a function of polymer molecular weight of the enthalpy of the transition, ΔH (open circle), the temperature corresponding to the maximum in DSC enthalpograms, T_m (full circle), and the cloud point, CP (full triangle), for aqueous solutions of poly(2-isopropyl-2-oxazolines). Polymer concentration: 1.0 g L^{-1}. Heating rate: 60 °C min^{-1}.

5.2.3 Pressure perturbation calorimetry

PPC scans were carried out next with solutions of $PIPOZ_n$-OH (n = 17, 21, 41 and 50) in H_2O and in D_2O, yielding the changes with temperature of the thermal expansion coefficient, α_{pol}, shown in Figure 7 (bottom), in the case of $PIPOZ_{50}$-OH . The plot can be divided in three temperature ranges. Below T_{onset}, for 15 °C < T < 42 °C, α_{pol} remains constant. It undergoes a sharp increase, reaches a maximum for T_{peak}= 45.0 °C, and gradually decreases as the temperature further increases. PPC scans recorded with solutions of the same polymer in D_2O presented the same features (Figure 7, bottom), with slight differences in the temperatures of the maximum of α_{pol}, that correspond to the slight shifts of T_{onset} and T_{max} already noted in the DSC scan (Figure 7, top). The changes in volume of the solvation layer of the polymer (ΔV), corresponding to the collapse of the chain and chain aggregation during the phase transition, can be extracted from PPC scans by integration of the changes in α_{pol} as a function of temperature, assuming that the intrinsic volume occupied by a polymer chain remains constant. The volume change, expressed as $\Delta V/V$ in percent of the partial volume of the polymer (eq 2), is taken as the area defined by the peak of the PPC scan and a progress baseline (dashed line, Figure7, bottom) drawn from projections of the baselines in the pretransition and posttransition regions. Values of $\Delta V/V$ for PIPOZ-OH samples in H_2O, D_2O, and aqueous NaCl are listed in Table 2. Comparing samples of PIPOZ-OH of increasing molecular weight, we observe that the solvation volume change during the phase transition is extremely

sensitive to the molecular weight of the oligomer, nearly doubling in value for solutions of $PIPOZ_{50}$-OH, compared to the solutions of the shortest oligomer, $PIPOZ_{17}$-OH.

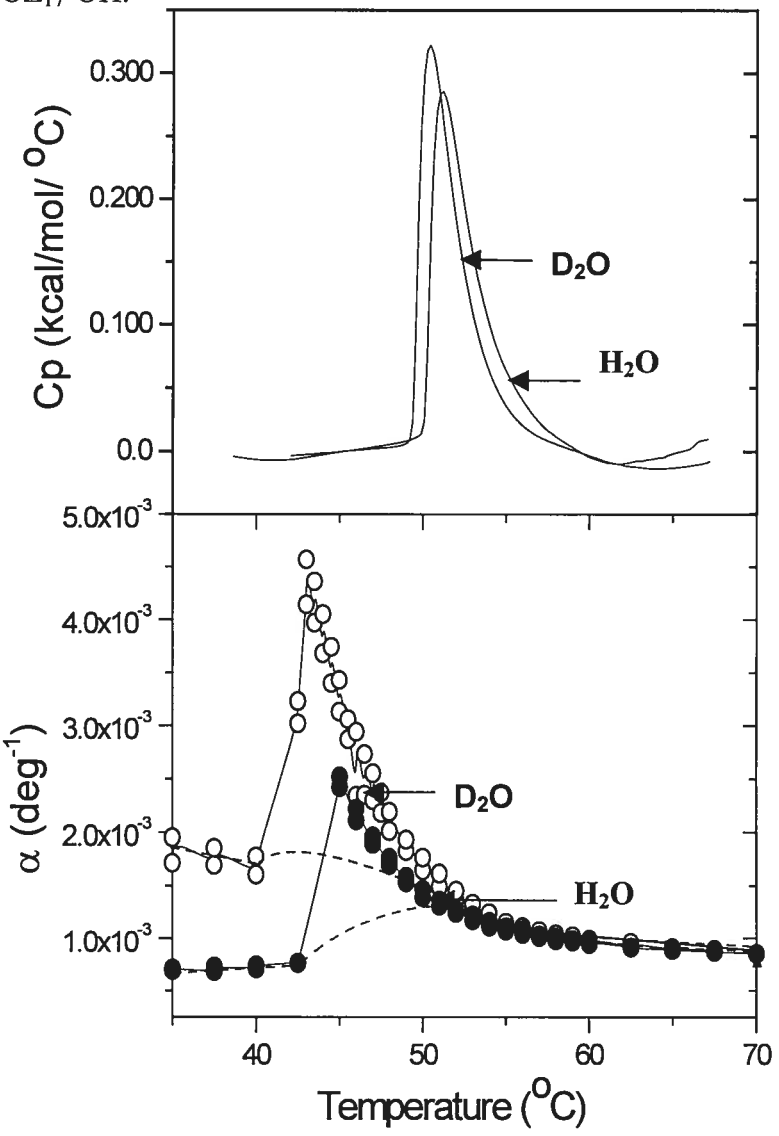


Figure 7 : (top) Microcalorimetric endotherms for a solution of $PIPOZ_{50}$-OH in H_2O and in D_2O. Polymer concentration 1 g L^{-1}. (bottom) Temperature dependence of the coefficient of thermal expansion (α_{pol}) of $PIPOZ_{50}$-OH in H_2O (full circle) and in D_2O (open circle). Polymer concentration: 5 g L^{-1}. The doted line corresponds to the progress baseline.

This significant effect corroborates the sensitivity to molecular weight of the enthalpy of transition (vide supra) and may be taken as added evidence that the number of hydrogen bond sites along the chain increases with molecular weight.

Considering next the values of $\Delta V/V$ recorded in H_2O and in D_2O, we note that for all samples, the volume change in D_2O is larger by ~ 50 % than the value recovered from measurements in H_2O. The enhanced change in hydration volume in D_2O, compared to H_2O, results from a combination of two effects: (1) the PIPOZ coils may adopt a more extended conformation in D_2O, resulting in a higher level of ordering of the D_2O molecules bound to the polymer chain and hence a larger volume of bound water and (2) since D_2O is a more "structured" solvent than H_2O, the water molecules expelled from the hydration layer occupy a larger volume of bulk solvent. The changes in the volume of hydration of the polymer during the phase transition are also affected by the presence of NaCl: the values of $\Delta V/V$ increase with increasing salt concentration (Table 2), reflecting salt-induced changes in the hydration layer and in the polymer chain stiffness in solutions of temperature lower than the transition temperature.

6. Conclusions

As noted in the Introduction, the repeat unit of poly(2-isopropyl-2-oxazoline) is a structural isomer of the repeat unit of poly(N-isopropylacrylamide). These two polymers share a number of characteristics when dissolved in water: they both undergo a reversible heat-induced phase transition when brought to a temperature beyond a critical value. This temperature, detectable by changes in the transmission of the solution, or more accurately measured by DSC, depends on salt concentration and is different for solutions

of the polymers in H_2O and D_2O. The phase transition of PNIPAM has been studied in great detail.[42] As the PIPOZ samples investigated here are monodisperse and of low molecular weight, we chose to compare their properties to those of PNIPAM oligomers, taking values from published reports. Microcalorimetric studies carried out independently with NIPAM oligomers bearing a carboxylic acid end-group revealed that solutions of PNIPAM (M_n 1,600, 2,200, 2,400, and 3,500) have a transition temperature slightly higher than solutions of higher molecular weight PNIPAM (33.3 °C vs 31.5 °C) with a transition enthalpy of 0.45 kcal mol^{-1} L^{-1}.[43, 44] These studies concur to indicate that, although the transition temperature, T_m, is only slightly higher for NIPAM oligomeric solutions, compared to T_m of solutions of PNIPAM samples of higher molecular weight and wider polydispersity, [33, 45] the transition peaks are wider and the transition enthalpies weaker, in the case of NIPAM oligomers. The latter two features are observed in our study of PIPOZ, suggesting that they are related to molecular weights of the samples, rather than to their chemical constitution.

There are however several differences in the thermodynamic traits of aqueous solutions of PNIPAM and PIPOZ oligomers (Table 2). First, the molecular weight sensitivity of T_m is pronounced in the case of PIPOZ, and not PNIPAM. Second, the transition temperature of aqueous PNIPAM is higher in D_2O, compared to H_2O, whereas aqueous PIPOZ solutions exhibit the opposite effect. The latter situation seems to be a more common occurrence: it has been reported for solutions of hydroxypropyl cellulose[46] and poly(N-vinylcaprolactam),[35] two polymers that possess a cloud point in water. The enthalpy of transition is higher in D_2O, compared to H_2O, for solutions of PNIPAM or PIPOZ, but the enhancement in D_2O is significantly larger in the case of PIPOZ. Third,

thermograms recorded with PNIPAM solutions present a negative change in ΔC_p ($\Delta C_p = C_p(T > T_m) - C_p(T < T_m) = -63$ J mol^{-1} K^{-1}), while $\Delta C_p \sim 0$ for PIPOZ. A negative change of C_p was reported also in studies of the phase transitions of various pluronic-type block copolymers.[47] Such a negative heat capacity change during the phase transition may be taken as an indication of diminished interaction between water molecules and polymer chains, before and after the transition.

7. Acknowledgments

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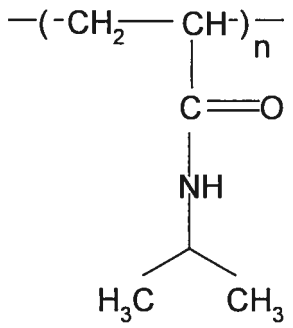
CHAPTER 3

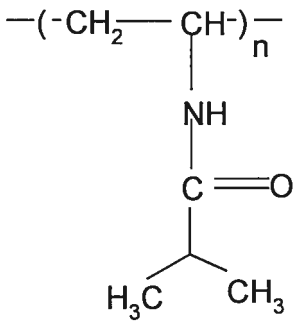
A Study by Differential Scanning Calorimetry and Pressure Perturbation Calorimetry of the Thermal Properties of poly(N-vinylisobutyramide) in water.

1. Introduction:

Poly(N-vinylisobutyramide)(PNVIBA) is a water soluble polymer and its solubility in water responds sharply to temperature changes [1,2]. As mentioned earlier in the introduction chapter, the solubility-temperature relationship of water soluble polymers having alkyl groups in the side chain [3], or at the end of the polymer chain [4], depends on the hydrophilic-hydrophobic balance in the polymer structure. The strength of hydration of the polymer plays a dominant role in the phase transitions [5]. The transitions for such polymers in aqueous solutions occur by dehydration, the strengthening of hydrophobic interactions among the side chains [6, 7, 8, 9] and the disruption of the hydrogen bonds formed between the polymer and water. Aqueous solutions of PNVIBA (0.1-5 g L^{-1}) show a lower critical solution temperature (LCST) at approximately 39 ^{0}C [2]. The solution becomes turbid above the LCST and the polymer is reversibly solubilized below the LCST. It is well known that PNIPAM shows an LCST at approximately 32 ^{0}C [10, 11, 12], which is lower than that of PNVIBA. Both compounds have a similar chemical composition but a reversed amide linkage (figure 1) and a quite similar hydrophobic-hydrophilic balance. In the case of PNIPAM, the carbonyl group of the amide function is linked to the polymer backbone, whereas in PNVIBA the amide function is linked to the polymer backbone by a carbon-nitrogen bond, the carbonyl thus is further removed from the main chain. The properties of the two polymers have been compared in recent studies of Akashi and coworkers using ^{1}H NMR relaxation [13] and pressure dependant solubility analysis [14].



Poly(N-isopropylacrylamide)



Poly(N-vinylisobutyramide)

Figure 1 : Chemical structure of the repeat units of poly(N-isopropylacrylamide) (PNIPAM) and poly(N-vinylisobutyramide) (PNVIBA).

Furthermore, they reported that these two polymers have similar chemical groups in their repeat units as homopolypeptides, such as polyleucine and polyisoleucine [14, 15]. The dependence of the thermosensitive properties on their chemical structure is of great scientific interest. The higher transition temperature of PNVIBA can be explained by the fact that the hydration of the hydrophilic groups of this polymer at lower temperatures is stronger than in PNIPAM. Indeed, the changes in hydration layer are the major forces involved in the phase transition of polymers aqueous solutions. Few techniques were found to be sensitive to solute hydration in dilute aqueous solutions, so that the detection of the hydration contribution for such solute domain was difficult. Recently, Kujawa and Winnik have introduced an effective technique, the pressure perturbation calorimetry (PPC), which can be used to determine the hydration properties of a polymer. They have studied the hydration properties of PNIPAM in H_2O and D_2O [24] and they have found

that the phase transition was accompanied by an increase in the partial volume of the hydrated PNIPAM in both solvents. This increase was significantly higher in D_2O, compared to H_2O. The phase transition of the polymers can be influenced by the presence of salt [16], surfactant [17], and by the nature of the solvent [18]. These polymers that respond with large properties changes to small physical or chemical stimuli are called intelligent polymers as defined by Hoffman [20, 21, 22].

In order to understand the differences between the poly(N-vinylisobutyramide) and poly(N-isopropylacrylamide), the solutions properties of PNVIBA were studied in details as reported in this chapter. Two calorimetric techniques were used: the differential scanning calorimetry (DSC) allowed us to determine the phase separation temperature and the enthalpy of the transition, pressure perturbation calorimetry (PPC) was used to measure the thermal expansion coefficient of the polymer, α_{pol}, as function of the temperature, the effect of pressure on the phase transition temperature T_m, and the volume changes, ΔV, associated with the phase transitions. The results obtained were compared to the corresponding results for poly(N-isopropylacrylamide) (PNIPAM) solution reported elsewhere [15, 23, 24, 25].

2. Experimental section

2.1 Materials

Homopolymers of PNVIBA (M_w=18.7kDa, PDI=1.95, M_w=10kDa, PDI=2) were received from laboratory of professor Akashi M.(Kagoshima University, Kagoshima Japan) as a gift and were prepared as described previously [1]. PNIPAM (Mn = 13kDa, PDI= 1.1) was prepared in our laboratory. Water was deionized with a Millipore Milli-Q

water purification system. Sodium chloride and deuterium oxide (99.8 %) were purchased from Sigma-Aldrich Chemicals.

2.2 Synthesis and characterization of PNVIBA (Table 1)

PNVIBA were prepared by radical polymerisation by Akashi and coworkers in Japan using the same procedure described elsewhere [1].

Polymer	M_n[a] ($g\ mol^{-1}$)	M_w[a]	M_w/M_n[a]	DP[a]	T_{cp} (^0C)[b]
$PNVIBA_{44}$	5,028	9,800	2.00	44	40.2± 0.2
$PNVIBA_{82}$	9,600	19,200	1.95	82	38.9± 0.2
$PNIPAM_{115}$	13,000	14,300	1.10	115	------

[a] from GPC measurements;
[b] from turbidity measurements (polymer concentration 2 $g\ L^{-1}$)

Table 1 : Characteristics of the poly(N-vinylisobutyramide).

They were characterized by NMR spectroscopy. NMR spectra were recorded on a Bruker ARX-400 400 MHz spectrometer in $CDCl_3$. ^1H NMR ($CDCl_3$, δ) 0.96 (s, [**$(CH_3)_2$**CH-], 2.4 (m, [$(CH_3)_2$***C**H*-], 4.4 (d, (CH_2-**CH**-). The molecular weights of the polymers were determined by gel permeation chromatography (GPC) using Schimadzu LC-6A system equipped with a differential refractometer detector (Shodex SE-51) and a Shodex column (AD-80M/S) eluted with a DMF solution containing 10mM LiBr at a flow rate 0.8 mL min^{-1} at 40^0C. Calibration was performed with poly(ethylene oxide) standards. Cloud

points were determined by spectrophotometric detection of the changes in turbidity (λ = 500 nm) of aqueous polymer solutions (2.0 g L^{-1}) heated at a constant rate in a UV cell. The turbidity measurements were performed by Suwa, Morishita, Kishida and Akashi and the results have been reported previously [28]. UV Visible spectra were obtained using a Hitachi model 200-20 spectrophotometer in a 1.0 cm path-length.

2.3 Instrumentation

2.3.1 Differential scanning calorimetry (DSC)

DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc) at an external pressure of ca. 180 kPa. The cell volume was 0.52 mL. The heating rate was 1.0 ^{o}C min^{-1} and the instrument response time was set at 5.6 s. Data were corrected for instrument response time and analyzed using the software supplied by the manufacturer. The polymer concentration ranged from 1 to 5g L^{-1}.

2.3.2 Pressure perturbation calorimetry (PPC)

Measurements were made using a standard VP-DSC instrument equipped with a PPC accessory. The total fill sample and reference cells have identical volumes of 0.52 ml. Filling chamber contains a sensor which transmits data to computer for storage . Pressure in the chamber is alternated between P_1 and P_2, using a controller source operating at selected excess pressure from 0 to 5.5 atm and this pressure controller connects the PPC cell with nitrogen tank. The solution is placed in the sample cell and the solvent in the reference cell. The measurements were taken in the temperature range 25-80 using high gain and a filtering period of 1 second. The polymer concentration was

5 .0 g L^{-1}. Control experiments were performed, water (sample cell) *vs* water (reference cell), H_2O *vs* D_2O, D_2O *vs* D_2O, NaCl in water *vs* NaCl in water, and water *vs* NaCl in water. The PPC has been described in details in chapter one and in appendix A.

3. Results and discussion

3.1 High-Sensitivity differential scanning calorimetry

From a DSC thermogram, one can extract three thermodynamics parameters T_{onset} (onset of the transition) (shown in figure 2), T_{max} (temperature that corresponds to the highest heat capacity) and ΔH, the enthalpy of the transition.

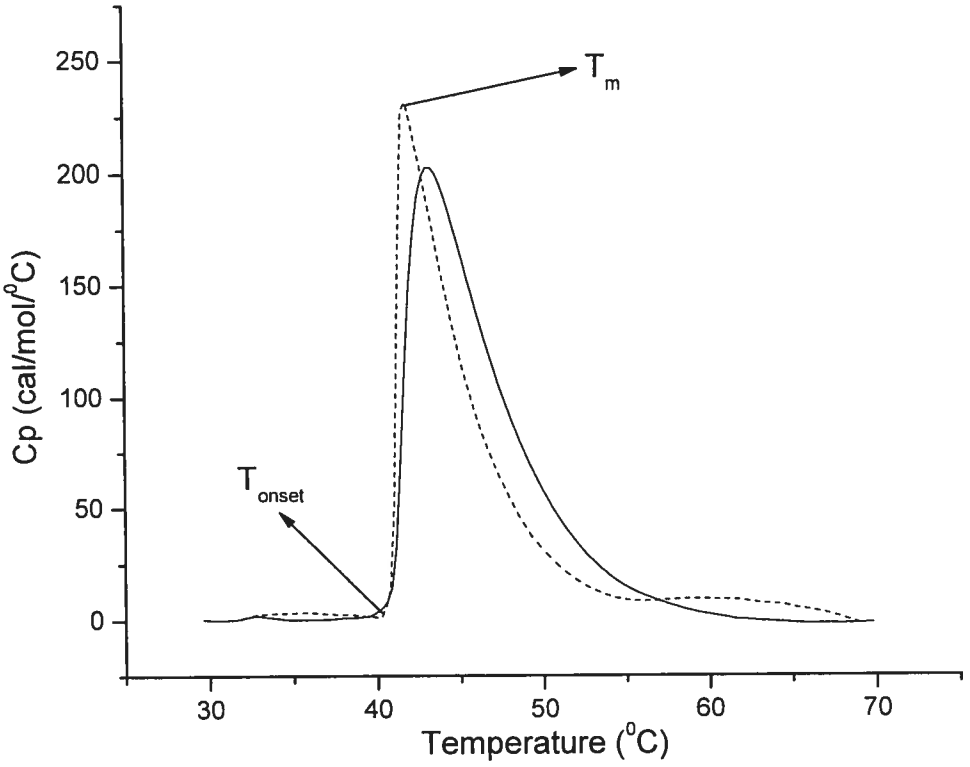


Figure 2 : Microcalorimetric endotherms for aqueous solutions of $PNVIBA_{44}$ (solid line) and $PNVIBA_{82}$ (dashed line) samples in water. Polymer concentration: 1.0 g L^{-1}. Heating rate: 60 °C h^{-1}.

The thermograms obtained for two samples of PNVIBA with different molecular weights (table 1) are presented in figure 2; they represent the changes as a function of temperature of the partial excess heat capacity of the two polymers solutions (1 g L^{-1}). The two thermograms are almost identical; they are broad, and endothermic. The transition observed is reversible and the thermograms were reproducible after repeating the scan three times at the same heating rate ($1^0C.min^{-1}$). Polymer with smaller molecular weight showed a higher transition temperature and a broader endotherm; this result confirms previous conclusions based on turbidimetry measurements [26, 27, 28]. The enthalpy of the transition above depends on the molecular weight and a small increase of ΔH with increasing molecular weight (table 2) was detected.

Table 2 : Thermodynamic parameters of the poly(N-vinylisobutyramide) studied.

Polymer	Solvent	T_{onset} (°C)[a] (DSC)	T_{max} (°C)[a] (DSC)	ΔH (kcal mol^{-1})[a] (DSC)	T_{max} (°C)[b] (PPC)	$\Delta V/V$ (%)[b]
$PNVIBA_{44}$	H_2O	39.6 ± 0.2	43.0 ± 0.3	1.22 ± 0.01	42.0 ± 0.3	1.20
	D_2O	39.6 ± 0.2	43.2 ± 0.3	1.38 ± 0.01	42.3 ± 0.3	1.50
	NaCl (0.5M)	31.3 ± 0.2	35.0 ± 0.3	1.53 ± 0.01	35.1 ± 0.3	1.30
	NaCl (1.0M)	20.7 ± 0.2	31.0 ± 0.3	1.65 ± 0.01	30.6 ± 0.3	1.10
	H_2O[d]	39.2 ± 0.2	42.1 ± 0.3	1.41 ± 0.01	42.0 ± 0.3	1.10
$PNVIBA_{82}$	H_2O	40.0 ± 0.2	41.8 ± 0.3	1.22 ± 0.01	42.0 ± 0.3	0.93
	D_2O	40.0 ± 0.2	42.0 ± 0.3	1.44 ± 0.01	42.3 ± 0.3	1.20
PNIPAM	H_2O	24.5 ± 0.2	32.6 ± 0.3	0.80 ± 0.01	32.0 ± 0.3	0.90
	D_2O[c]		34.3± 0.3	1.50 ± 0.01	34.3 ± 0.3	1.32
	NaCl (0.5 M)	18.4± 0.2	25.6 ± 0.3	1.20 ± 0.01	32.0 ± 0.3	1.00
	NaCl (1.0 M)	13.6 ± 0.2	20.1 ± 0.3	1.37 ± 0.01	26.0 ± 0.3	1.20

[a] from DSC measurements (polymer concentration: 1.0 g L^{-1})
[b] from PPC measurements (polymer concentration: 5.0 g L^{-1})
[c] from reference 24
[d] polymer concentration: 5.0 g L^{-1}

The thermograms observed for two different concentrations of PNVIBA (M_n = 5 kDa) are shown in figure 3. Upon dilution of the polymer solutions, the endothermic peak shifted slightly to higher temperature, the enthalpy of the transitions decreased (Table 2), and the peak became sharper (Figure 3).

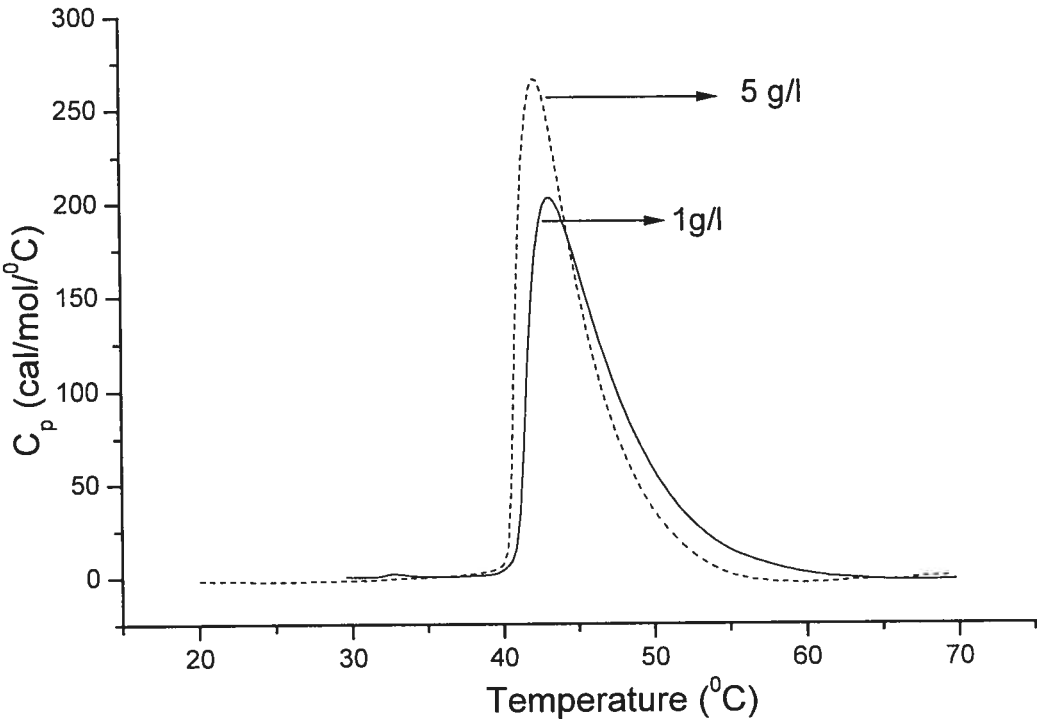


Figure 3 : Microcalorimetric endotherms for aqueous solutions of $PNVIBA_{44}$ with two different concentrations: Polymer concentrations: 1g L^{-1} (solid line) and 5g L^{-1} (dashed line) in water. 1.0 g L^{-1}. Heating rate: 60 °C h^{-1}.

The phase transition of PNVIBA is also influenced by the presence of NaCl: first, the transition temperature decreased with increasing NaCl concentration (Figure 4).

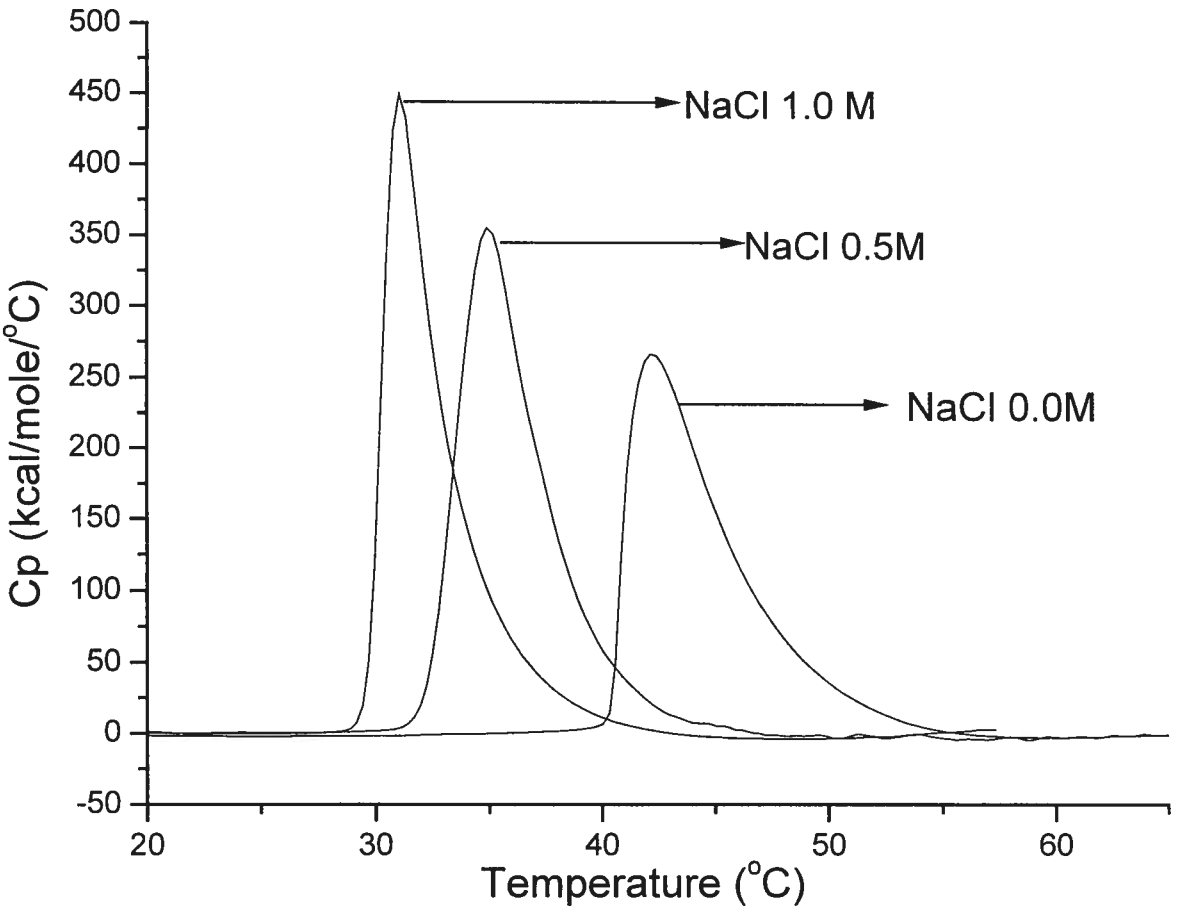


Figure 4 : Microcalorimetric endotherms for aqueous solutions of $PNVIBA_{44}$ in the presence of different concentrations of salts. Heating rate: 60 °C h^{-1}.

NaCl is known to have a salting-out effect. Salting-out (structure making) strengthens hydrophobic interactions, favoring the formation the globular structures (collapsed state); in other words, the hydration layer was weakened by the addition of salting out ions, because as increasing the salt concentration the number of hydrogen between water molecules increases (water molecules are more ordered). This results in a decrease of the number of hydrogen bonds between water molecules and the polymer amide groups in

the hydration shell around the polymer chain. The number of strong hydrophobic interactions between the polymer functionalities increases. The chains collapse at a lower temperature in the presence of NaCl. Second, the enthalpy of transition increases with increasing the salt concentration (table 2), which may be the result of structural changes in the cluster of water molecules due to the structure making properties of NaCl. Water molecules will be more ordered and the energy to break the hydrogen bonds between water-water and water polymer will become higher.

DSC thermograms recorded for polymers of different molecular weight in light and heavy water were recorded (Figure 5).

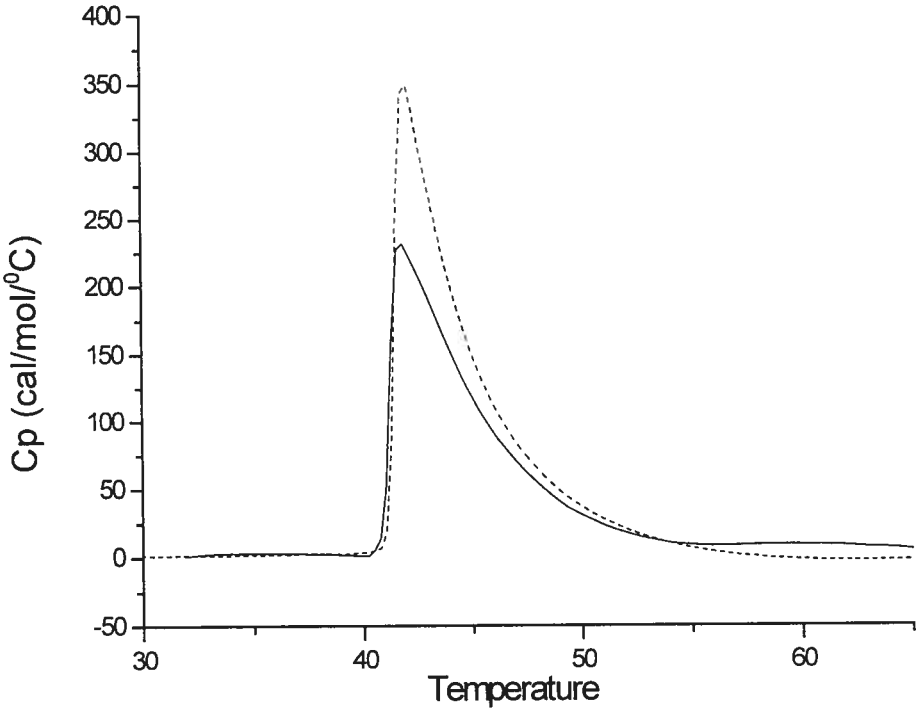


Figure 5 : Microcalorimetric endotherms for a solution of $PNVIBA_{82}$ in H_2O (solid line) and in D_2O (dashed line). Polymer concentration 1 g L^{-1}. Heating rate: 60 °C h^{-1}.

A subtle increase in the transition temperature is observed when H_2O is replaced by D_2O. It is believed that this behavior is due to the hydrophobic interactions, which play a crucial role and seem to be stronger in D_2O than H_2O [24]. The enthalpy of the transition is higher in D_2O than in H_2O, presumably due to the heat capacity of D_2O which is higher than that of H_2O.

3.2 Pressure perturbation calorimetry

PPC scans were carried out with solutions of $PNVIBA_n$ (n = 44 and 82) in H_2O and in D_2O; the change with temperature of the thermal expansion coefficient, α_{pol}, was recorded. In order to interpret the scans, three temperature regions will be considered : below the phase transition, around the phase transition and above the phase transition. Shown in figure 6 are the PPC scans of PNVIBA of two different molecular weights. Below the phase transition , $25 < T < 39\ ^0C$, no change of the α_{pol} with temperature is observed. Around the transition, $39 < T < 50\ ^0C$, α_{pol} increases sharply, reaching a maximum at T_{max}, followed by a decrease reaching a value nearly equal to that recorded before the transition temperature. After the transition $50 < T < 80\ ^0C$, the values of α_{pol} remain constant up to $65\ ^0C$ then increase slightly with increasing temperature; this behavior is different from that of PNIPAM [24]. The solutions of the latter exhibit a *decrease* in α_{pol} in the same range of temperature.

The change in volume $\Delta V/V$ of the solvation layer of the polymer during the temperature- induced collapse of the chain can be obtained by integrating the changes in α_{pol} as function of temperature. The values of $\Delta V/V$ (table 2) vary depending on the

molecular weight: a decrease of $\Delta V/V$ is detected with increasing the molecular weight (table 2).

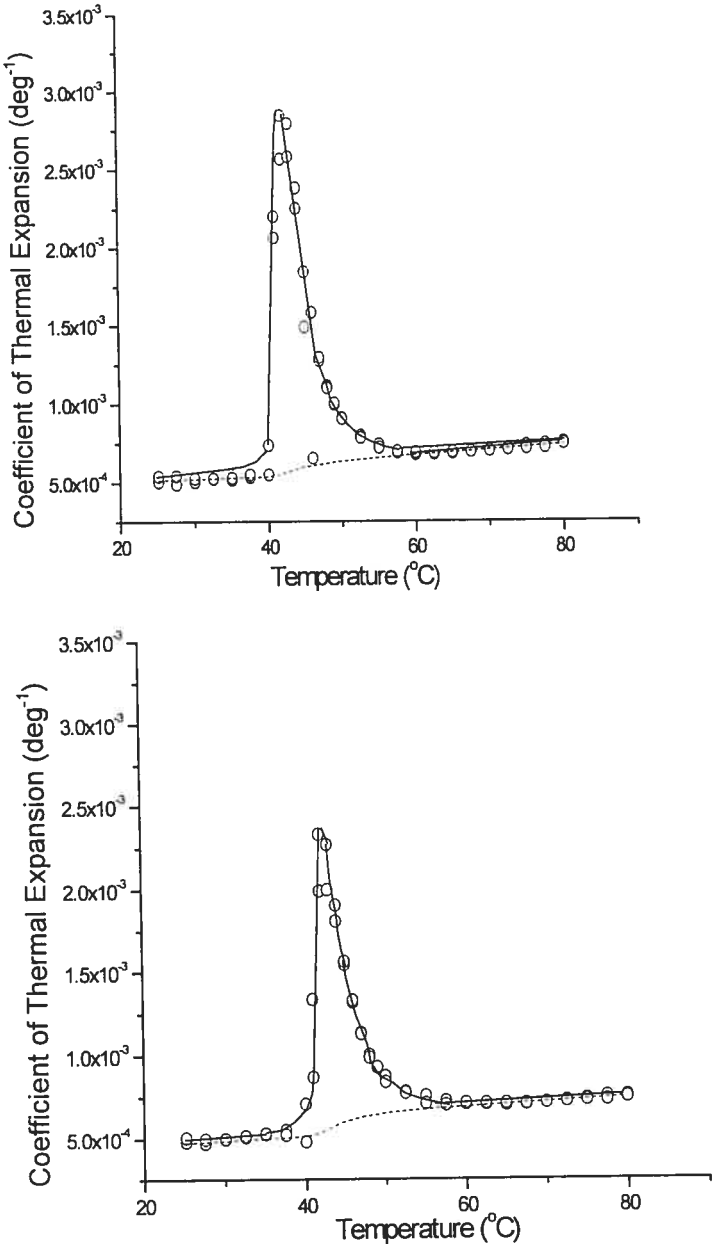


Figure 6 : Temperature dependence of the coefficient of thermal expansion (α_{pol}) of $PNVIBA_{44}$ (Top) and of $PNVIBA_{82}$ (bottom). Polymer concentration: 5 g L^{-1}. The doted line corresponds to the progress baseline

This large volume change (27%) may simply indicate that the water molecules around the high molecular weight polymer chains are more ordered, and some of the hydrogen bonds between the polymer and water are replaced with some intermolecular polymer-polymer interactions, so that at the transition temperature the water molecules released from the hydration layer of the polymer occupy a smaller volume of solvent (in the case of high molecular weight).

The influence of NaCl on the transition temperature and on the volume change of the solvation layer was also investigated (figure 7). As detected by DSC, the T_m decreases with increasing salt concentration (table1). In general, the effect of salts may result from direct interactions of salt ions with the side chain polar groups (such as amide) or the effects on the hydrogen bonding ability of water. Moreover, the presence of salts affects enormously the changes in the volume of hydration of the polymer; increasing the concentration of NaCl from 0.5 to 1.0 M, $\Delta V/V$ decreases by 20% (table 1), which may be explained by dehydration process.; in other words, the hydration layer is weakened by the addition of salting out ions. As the salt concentration increases, the number of hydrogen bonds between water molecules increases (water molecules are more ordered), and there are fewer water molecules available for the hydration shell around the polymer chain, enhancing the hydrophobic interaction and triggering chain collapse at lower temperature, fewer water molecules will be expelled from the polymer hydration layer and consequently $\Delta V/V$ is smaller.

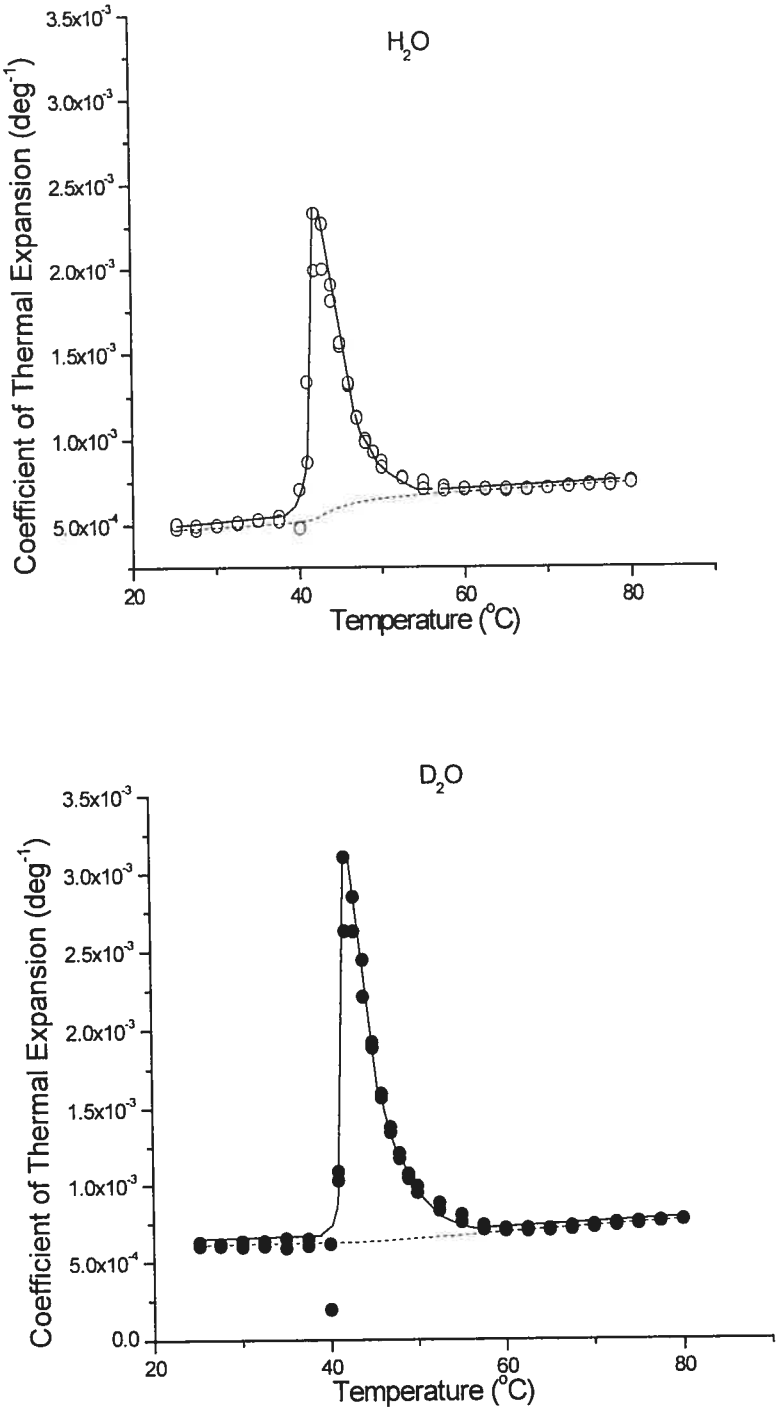


Figure 7 : Temperature dependence of the coefficient of thermal expansion (α_{pol}) of $PNVIBA_{82}$ in H_2O (Top) and in D_2O (bottom). Polymer concentration: 5 g L^{-1}. The doted line corresponds to the progress baseline

Presented in figure 8 are the PPC scans obtained for the polymers dissolved in H_2O and D_2O.

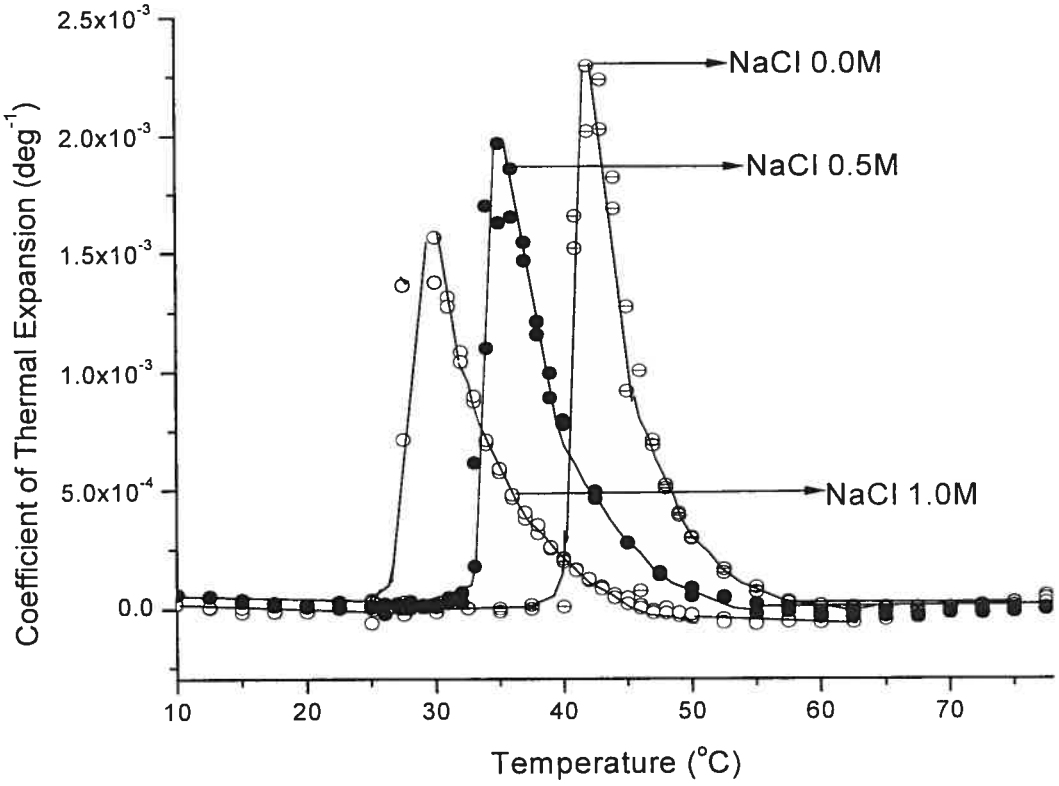


Figure 8 : Temperature dependence of the coefficient of thermal expansion (α_{pol}) of $PNVIBA_{44}$ in the presence of different concentration of salts. Polymer concentration: 5 g L^{-1}.

The changes in T_m (table 1) are in good agreement with the DSC results. Comparing the values of $\Delta V/V$ in H_2O and D_2O (table 1), one can see a larger volume change in D_2O (~30%). The explanation of this behavior is that the PNVIBA coils are more extended in D_2O, this will result in a higher level of ordering of D_2O associated with a polymer chain and a larger volume of interacting water, so that the water molecules expelled from the

hydration layer at the transition temperature will occupy a large volume of solvent in the case of D_2O.

4. Conclusion

The results of this study have shown that PNVIBA aqueous solutions undergo a phase separation above a critical temperature. In order to compare PNVIBA to PNIPAM, a microcalorimetric study of a PNIPAM of similar molecular weight (Mn ~13 kDa) (Table1) was carried out. The phase transition of PNIPAM has been studied by many techniques in details [12, 15, 24] . Compared to PNIPAM, the phase separation of PNVIBA solutions occurs at higher temperature (~17%) and is accompanied by larger ΔH values. This enthalpy originate mainly from the disruption of hydrogen bonds between water molecules and the side chain of the polymer. A larger value of ΔH of PNVIBA may simply indicate that the intermolecular hydrogen bonds are more tight in PNVIBA than in PNIPAM or that the hydration layer to the side chains of the PNVIBA is stronger than in PNIPAM; this could be due to the direction of the amide bond in the side chain, which is the main difference in structure between those two polymers. It would seem that the hydrogen bonds are more favorable when the NH is closest to the main chain and the CO is further removed and closer to the isopropyl group. From PPC results (table 1), the volume of the solvation layer $\Delta V/V$ of PNVIBA was found to be larger by ~30% compared to PNIPAM. Water molecules around the PNIPAM chains are more ordered, occupying a smaller volume, and some of the hydrogen bonds between the polymer and water are replaced with some intermolecular polymer-polymer

interactions, so that at the transition temperature the water molecules released from the hydration layer of the polymer occupy a smaller volume of solvent.

It has been reported that solutions of PNIPAM with low molecular weight have a temperature transition slightly higher than that of higher molecular weight [19, 25]. This latter property was observed in our study for the PNVIBA denoting that the T_m is related to their chemical composition rather than the molecular weights of the samples; in fact one can detect a slight variation in the T_m and the enthalpy of the transition with the molecular weight for PNVIBA and PNIPAM. In addition, a similar concentration dependence on the T_m were observed for both polymers; the T_m decreases with increasing concentration. Also, replacing H_2O by D_2O has the same effect on the T_m of PNIPAM and PNVIBA; the transition temperature is always higher in D_2O compared to H_2O. The effect of salts on the transition temperature of these polymers also was found to be the same; the T_m decreases upon increasing the salt concentration but the change is significantly larger in the case of PNIPAM. This could be related to many possible factors. The salting-out anions are water structure makers and as a result, they strengthen hydrophobic interactions among the chains of the polymers, thereby the hydration of the side chains of the polymers is weakened and the collapse occurs at lower temperature.

In conclusion, we can say that the differences in the solution properties of the two polymers originate primarily from differences in the strength of hydration and of the hydrophobic interactions among the side chains. This means that both intermolecular and intramolecular interactions in solution play a crucial role in controlling the phase separation of these polymers.

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CHAPTER 4

Conclusion

1. Conclusion

During the past few decades, significant advances have been made in polymeric drug delivery technology. In the future, drug delivery will need more sophisticated and diverse formulations for existing drugs as well as for new drugs. In this study, our main goal was the determination of thermodynamic properties of novel thermosensitive, biodegradable, and non toxic polymers. We believe that these polymers will have an important role in the development of controlled-release formulations. Poly(2-substituted oxazoline) block copolymers have a number of unique and useful properties that are ideal for controlled drug delivery. The properties of the solution phase transition at well-defined temperatures and biodegradability make block copolymers ideal delivery vehicle for various drugs. Kataoka et al have already prepared end functionalised block copolymers using heterotelechelic poly(ethylene glycol) (PEG) as macroinitiator [1]. For example, PEG having an acetal group at one end and a methane sulfonate at the other end is found to initiate the polymerization of 2-methyl-2-oxazoline to form the acetal-PEG-POx block copolymers [1]. In addition, it has been found that by changing the alkyl constituent in the side chain, new characteristics can be given to oxazoline polymers [2, 3].

In this work, 2-isopropyl-2-oxazoline was chosen as monomer to obtain a novel thermosensitive polymer with isopropyl group at the side chain, just like in poly(N-isopropylacrylamide). The resulting polymer responds to temperature changes in physiological conditions. This temperature was found to be strongly related to the molecular weight of the polymer, on the hydrophilic-hydrophobic balance, the concentration of the solution, the solvent used, and the presence of salts. Our fundamental

study of poly(2-isopropyl-2oxazolines) (PIPOZ) has resulted in a better understanding of some important features, such as the phase separation phenomenon, its occurrence, and the thermodynamic parameters influencing it. This will facilitate the development of new drug carrier systems and medical devices using the poly(2-isopropyl-2-oxazolines) (PIPOZ) and its block copolymers.

The solution properties of poly(N-vinylisobutyramide) (PNVIBA) has also been investigated. PNVIBA is a thermosensitive, water-soluble polymer. It is clear that the transition temperature of PNVIBA depends on the hydrophobic–hydrophilic balance of the polymer [4, 5], the molecular weight of the polymer, the concentration of the solution, the solvent and the presence of salts. The fact that the temperature transition of PNVIBA can be increased or decreased by combining hydrophilic and hydrophobic units into the polymer led to the preparation of thermosensitive hydrogels, the volume of which decreased above the phase separation by releasing absorbed water. These hydrogels made from crosslinked hydrophobic polymers have been widely applied in biomedical materials, pharmaceutical fields and analytical chemistry [6, 7, 8]. This study of PNVIBA solutions may help to develop new hydrogels based on PNVIBA and to use them in various fields.

We hope that our study offers a better understanding of these two polymers, introduces new researchers to this topic and gives the industrial sector how important are these thermosensitive polymers for developing new drug carriers devices.

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Appendix A

Equations involved in PPC analysis

1. **Single component systems:**

The second law of thermodynamics can be expressed in terms of entropy; so that the change in entropy dS is related to the heat change dQ for a reversible process by :

$$dS = \frac{dQ_{rev}}{T} \qquad (1)$$

Differentiation of equation 2 with respect to P at constant temperature gives

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = T\left(\frac{\partial S}{\partial P}\right)_T \qquad (2)$$

Using the Maxwell relation, $\left(\partial S/\partial P\right)_T = -\left(\partial V/\partial T\right)_P$, where V is the volume of the system and substituting into equation 3 gives :

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -\left(\partial V/\partial T\right)_P = -TV\alpha \qquad (3)$$

where α is the coefficient of thermal expansion of the system, $\alpha = 1/V\left(\partial V/\partial T\right)_P$.

The integration of equation 4 at constant temperature and over a small pressure range gives equation 5 where it is assumed that V and α are nearly invariant with a small pressure change:

$$Q_{rev} = -TV\alpha\Delta P \qquad (4)$$

2. Two components system:

When a polymer of mass m_p is dissolved in a solvent of mass m_s, the volume of the solution is given by equation 6 :

$$V_{total} = m_s V_s + m_p \overline{V}_p \qquad (5)$$

where $\overline{V}_p$ is the partial specific volume of the polymer in solution and V_s is the specific volume of pure solvent.

The differential of equation 6 with respect to temperature at constant pressure yields:

$$\left(\frac{\partial V_{total}}{\partial T}\right)_P = m_s\left(\frac{\partial V_s}{\partial T}\right)_P + m_p\left(\frac{\partial \overline{V}_p}{\partial T}\right)_P \qquad (6)$$

The substitution of the right hand side of equation 7 into equation 4 gives:

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -T\left[m_s\left(\frac{\partial V_s}{\partial T}\right)_P + m_p\left(\frac{\partial \overline{V}_p}{\partial T}\right)_P\right] \qquad (7)$$

After multiplying and dividing the first term in brackets by V_s and multiplying the second term by $\overline{V_P}$ we obtain:

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -T\left[m_s V_s \alpha_s + m_p \overline{V}_p \alpha_p\right] \qquad (8)$$

where α_s and α_p are the coefficients of thermal expansion of the solvent and the polymer, respectively.

The integration of this equation leads to the heat arising from pressure perturbation of the solution, which can be viewed as the sum of heats arising from the perturbation of the solvent and from the perturbation of the solvated polymer:

$$Q_{rev=} \; -T\left[m_s V_s \alpha_s + m_p \overline{V}_p \alpha_p\right] \Delta P \qquad (9)$$

The net heat ΔQ_{rev} is equal to the difference between equation 10 for the sample cell and for the reference cell :

$$\Delta Q_{rev=} -T\left[m_p\bar{V}_p\alpha_p - m_p\bar{V}_p\alpha s\right]\Delta P \qquad (10)$$

$$\alpha_p = \alpha_s - \frac{\Delta Q_{rev}}{T\Delta P m_p\bar{V}_p} \qquad (11)$$

The volume change ΔV during a transition is obtained by the integration of the coefficient of thermal expansion with temperature (*$\Delta V <<< V$, the value Δvis expressed as a percent of V):*

$$\frac{\Delta V}{V} = \int \alpha_p \, dT = \int \frac{1}{V}\left(\frac{dV}{dT}\right) dT \qquad (12)$$